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HAEMOPROTEUS BECKERI N. SP. AND TRYPANOSOMA
LAVERANI VAR. TOXOSTOMAE N. VAR. FROM THE BROWN
THRASHER (TOXOSTOMA RUFUM)

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Accepted for publication March 13, 1935

In September, 1934, a brown thrasher (*Toxostoma rufum*) was captured after having injured itself flying into the window of one of the college laboratories. Blood smears made from it and stained with Wright's showed the presence of *Haemoproteus* and *Trypanosoma*.

Twenty-five specimens each of male and female *Haemoproteus* were were drawn with the aid of a camera-lucida and then measured by means of dividers. For comparison, twenty-five specimens of each sex of adult *Haemoproteus columbae* were measured in a similar manner.

Haemoproteus beckeri n. sp.¹

(Figs. 1 and 2)

The adult female gametocytes measured from $10.84\ \mu$ to $13.70\ \mu$ in total length and from $2.42\ \mu$ to $3.99\ \mu$ in width, with a mean size of $12.0\ \mu$ by $3.27\ \mu$. The parasite has rounded ends with about 50 per cent of the specimens showing one end slightly broader than the other. The periphery of the parasite is in close contact with the host-cell membrane and in very close contact with the nucleus of the host cell. The contact is so close that the host-cell nucleus seems to lie in a groove or fold in the parasite. The cytoplasm stains dark blue and has many indistinct and diffuse vacuoles. The pigment granules are scattered, oval to rod-shaped and vary in size up to $0.75\ \mu$ by $1.50\ \mu$ and number 4 to 11 with a mean of 8.3. The nucleus stains pink, is spherical to oval and is off-center, away from the host-cell nucleus. The mean size is $1.90\ \mu \times 2.28\ \mu$.

The adult male gametocytes measured from $10.27\ \mu$ to $13.7\ \mu$ in total length and from $2.56\ \mu$ to $4.56\ \mu$ in width with a mean size of $12.06\ \mu$ by $3.36\ \mu$. The ends of the parasite are rounded although not as uniformly smooth as the female gametocytes. About 50 per cent show one end broader. The parasite is in close contact with the host-cell membrane and the nucleus, although the contact with the latter is not so pronounced as with the female gametocytes. There are many indistinct and diffuse vacuoles. The pigment granules are in the shape of short oval rods and are found grouped close together at the ends of the parasite. They number from 5 to 12 with a mean of 7.24 and vary in size up to $0.75\ \mu \times 1.5\ \mu$. The nucleus stains light pink, is elongate-ovate to sub-rectangular and lies opposite the nucleus of the host cell. The mean size is $2.88\ \mu \times 5.35\ \mu$.

¹ We take pleasure in naming this *Haemoproteus* after our estimable teacher and friend, Dr. Elery R. Becker, of Iowa State College.

It has been very difficult to decide as to the possible relationship between this *Haemoproteus*, from *Toxostoma rufum* (Linn.), and many other halteridia of the *H. columbae* type. Some that might be mentioned are *H. orizivora* Anschütz, 1909, from the Java sparrow, *Munia orizivora*; *H. danielewskyi* var. *tinnunculus* Wasielewski and Wülker, 1918, in the kestrel, *Cerchneis tinnunculus* L. and *H. lophortyx* O'Roke, 1930, from the California Valley quail, *Lophortyx californica* Shaw. Other authors have described or figured many examples of *Haemoproteus* parasites from birds in all parts of the world (See papers by Mazza and his colleagues (1927-1932) and Uegaki (1930). Only a few have been assigned specific names, probably because of the dual fact that many of the described species are hard to identify, due to the absence of good descriptions and illustrations, and that specific differences are hard to define. The question of host-specificity is open to further inquiry; although, if one may generalize from cross-infection experiments with other blood-inhabiting protozoa, it seems safe to assume that a rather strict host-specificity will eventually be shown to obtain here.

TABLE 1. Differential characters of three species of *Haemoproteus*
Female gametocytes

| | <i>H. columbae</i> | <i>H. lophortyx</i> * | <i>H. beckeri</i> |
|-------------------|--|---|---|
| Length and width | Max. 17.13 μ -4.85 μ Min. 13.7 μ -2.83 μ Mean 15.4 μ -3.67 μ | Max. 18 μ -2.5 μ Min. 1.5 μ | Max. 13.7 μ -3.99 μ Min. 10.84 μ -2.42 μ Mean 12.34 μ -3.27 μ |
| Ends | Not rounded One end not broader | Uniform rounded Nuclear end slightly broader | Rounded About half show one end broader |
| Parasite membrane | Close contact with periphery of host cell Not close contact with host-cell nucleus | Close contact with periphery of host cell Not close contact with host-cell nucleus | Close contact with periphery of host cell Very close contact with (overlapping) host-cell nucleus |
| Vacuoles | Indistinct and diffuse | Usually present, one the size of nucleus to two or more irregularly distributed | Many, indistinct and diffuse |
| Granules | Max. No. 45 Min. No. 20 Mean No. 33.04 Size up to 0.75 μ Spherical—scattered | Max. No. 52 Min. No. 15 Mean No. 24.25 Size up to 0.6 μ | Max. No. 11 Min. No. 4 Mean No. 8.3 Size up to— 0.75 μ x 1.5 μ Short rods, scattered |
| Nucleus | Spherical to oval Slightly off center Mean size— 2.11 μ x 2.3 μ | Spherical to oval Central next to host-cell nucleus Mean size— 1.5 μ x 2.5 μ | Spherical to oval Off center, away from host-cell nucleus Mean size— 1.9 μ x 2.28 μ |

* These data from O'Roke (1930).

H. beckeri differs from all the described forms, and tables 1 and 2 allow for comparison of this species with two well described forms, *H. lophortyx* O'Roke of the California valley quail and *H. columbae* Kruse of the pigeon.

The intermediate host of *H. beckeri* will probably be found to be a hippoboscid fly, although experimental proof is lacking at present. One of these flies (*Lynchia hirsuta* Ferris) is known to be the intermediate host of *H. lophortyx* and, according to O'Roke (1930), this species has been taken on the California thrasher, *Toxostoma redivivum redivivum*.

TABLE 2. Differential characters of three species of *Haemoproteus*
Male gametocytes

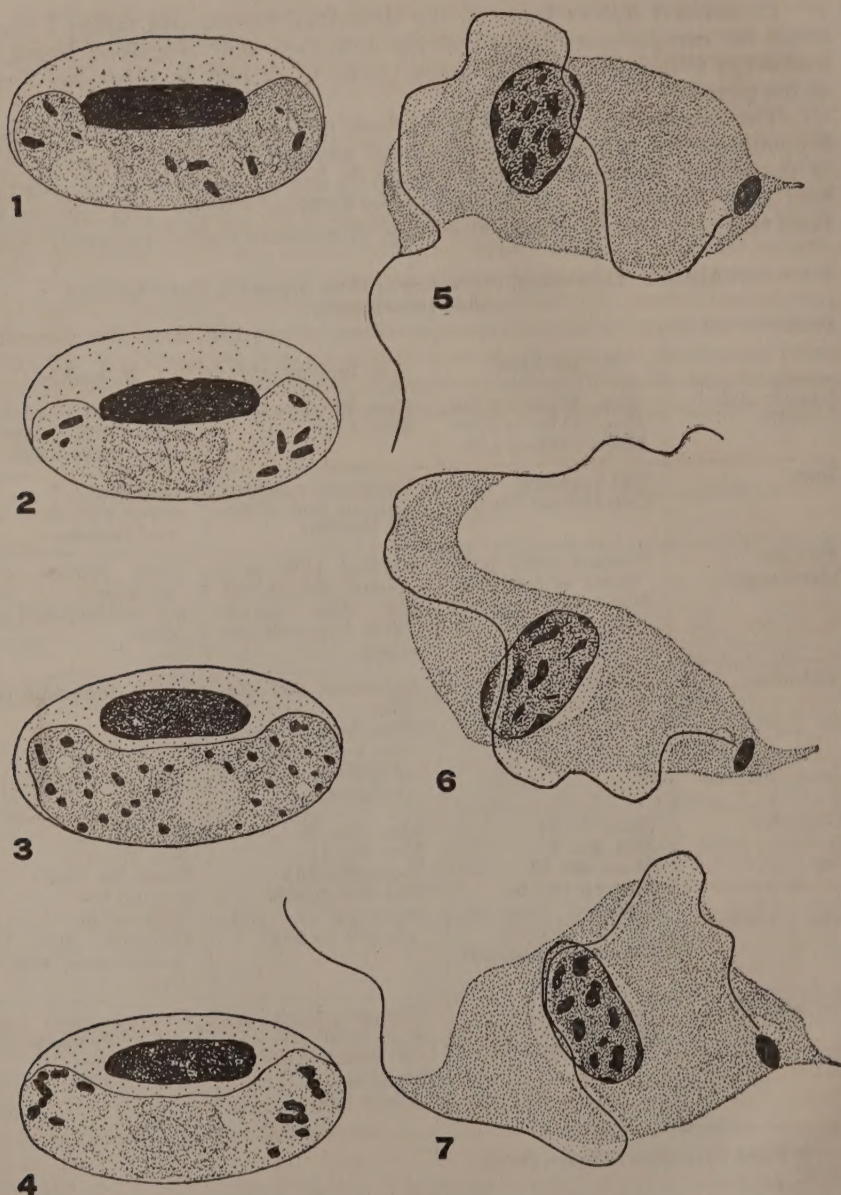
| | <i>H. columbae</i> | <i>H. lophortyx</i> * | <i>H. beckeri</i> |
|-------------------|--|---|--|
| Length and width | Max. $15.39\mu \times 4.28\mu$ Min. $11.9\mu \times 2.56\mu$ Mean $13.81\mu \times 3.19\mu$ | Max. $18\mu-2.5\mu$ Min. 1.5μ | Max. $13.7\mu-4.56\mu$ Min. $10.27\mu-2.56\mu$ Mean $12.06\mu-3.36\mu$ |
| Ends | Not rounded One end not broader | Uniformly rounded Nuclear end slightly broader | Rounded About half show one end broader |
| Parasite membrane | Contact with periphery of host cell Not close contact with host-cell nucleus | Contact with periphery of host cell Not close contact with host-cell nucleus | Close contact with periphery of host cell and host-cell nucleus |
| Vacuoles | Indistinct and diffuse | Indistinct and diffuse Often large one near one end with ring of granules around periphery | Many, indistinct and diffuse |
| Granules | Max. No. 17 Min. No. 7 Mean No. 12 Size up to 1.5μ Grouped close together at ends Generally spherical | Max. No. 39 Min. No. 11 Mean No. 19.6 Size 0.2μ to 0.8μ Spherical to rod-shaped or oval | Max. No. 12 Min. No. 5 Mean No. 7.24 Size up to— $0.75\mu \times 1.5\mu$ Grouped close together, short rods to oval |
| Nucleus | Elongate-ovate Mean size— $2.87\mu \times 5.82\mu$ | Elongate, ovate, always near one end of gametocyte Mean size— $1.5\mu \times 4.5\mu$ | Elongate-ovate to sub-rectangular Mean size— $2.88\mu \times 5.35\mu$ |

* These data from O'Roke (1930).

Trypanosoma laverani var. *toxostomae*

(Figs. 5-7)

Trypanosoma laverani was described by Novy and MacNeal (1905) from a single stained specimen taken from the blood of an American goldfinch. This specimen, it should be noted, did not have an apparent flagellum. (The photomicrograph of this specimen shows two erythrocytes



x ca 3900

- Fig. 1. Female Gametocyte of *Haemoproteus beckeri*.
 Fig. 2. Male Gametocyte of *H. beckeri*.
 Fig. 3. Female Gametocyte of *H. columbae*.
 Fig. 4. Male Gametocyte of *H. columbae*.
 Fig. 5-7. *Trypanosoma laverani* var. *toxostomae*.

lying in such a position that they might be covering the flagellum.) To the description of the stained specimen was added a rather detailed description of the cultural forms found in a blood culture.

At the same time Novy and MacNeal (1905) saw trypanosomes in the fresh blood of a brown thrasher (*Toxostoma rufum*), but none was observed on stained smears. Cultural forms of this trypanosome resembled those of *T. laverani* except that they had "a narrower posterior end and are narrower and more tapering, and possibly more coarsely granular." Agglutination was not so marked as in the cultural forms of *T. laverani*. The authors in the same paper express the opinion that the trypanosome from the brown thrasher is a new species.

In the blood of the brown thrasher collected, trypanosomes were found only after a diligent search. With great difficulty 25 specimens were found and measured as described for the haemoproteus. These resemble very much *T. laverani* as described by Novy and MacNeal. The comparison of size between the two may be seen in table 3.

TABLE 3. Comparison of trypanosomes from goldfinch and thrasher

| | <i>Trypanosoma laverani</i> from goldfinch (1) | <i>Trypanosoma laverani</i> var. <i>toxostomae</i> from thrasher (2) |
|---|--|--|
| Posterior end to center of parabasal body | 1.0 μ | 2.68 μ |
| Parabasal body to center of nucleus | 8.0 μ | 7.68 μ |
| Nucleus to anterior end of body | 10.0 μ | 8.32 μ |
| Length of body | 20.0 μ | 18.70 μ |
| Length of flagellum | | 7.90 μ |
| Size of nucleus | 4 x 2 μ | 4.5 x 2.3 μ |
| Length of parabasal body | 0.6 μ | 1.12 μ |
| Width of body at center of nucleus | 6.0 μ | 6.06 μ |

(1) Taken from Novy and MacNeal's single specimen.

(2) Average of 25 specimens.

The trypanosome from the thrasher is broad and spindle-shaped with a narrow, pointed posterior end. The large nucleus is centrally located, with its long axis perpendicular to the long axis of the cell. In some cases there are one or two large vacuoles situated close to the nucleus and usually a small vacuole just anterior to the parabasal body. The parabasal body varies from nearly circular to ellipsoidal in outline, the latter being most frequently found, and may be situated with its long axis either perpendicular or parallel to the long axis of the body.

From the description it will be seen that this trypanosome is very similar to *T. laverani*, but when the cultural forms described by Novy and MacNeal are considered there is a slight difference. Because of this difference the authors propose to designate the trypanosome from the thrasher *T. laverani* var. *toxostomae* n. var.

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AN ELECTRON TUBE NUL-INSTRUMENT FOR USE WITH THE GLASS ELECTRODE AND A DESCRIPTION OF A RUGGED TYPE OF GLASS ELECTRODE

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Accepted for publication April 11, 1935

The difficulties encountered in the measurement of glass electrode potentials has encouraged the development of a number of vacuum tube circuits, all of which accomplish the desired purpose with varying degrees of satisfaction. In a previous publication¹ a simple one-tube instrument was described which is satisfactory if the resistance of the glass electrode is not too great. A more sensitive instrument will be described here which is simple and inexpensive, embodying the advantages of a number of sets while it reduces the disadvantages to a minimum. The set is a simple resistance coupled direct current amplifier similar to the one employing four vacuum tubes described by Ellis and Kiehl².

A condenser is used in the grid circuit of the first tube which is charged and discharged by an electrometer switch of special design. By the use of a milliammeter, which takes the place of a galvanometer, the often troublesome drift is eliminated. The set reaches equilibrium within a few seconds so that measurements can be made immediately after the current is turned on. The precision is about 0.5 millivolts if the resistance of the cell being measured is not over 500 megohms.

More than a dozen of these sets are in use at this institution and elsewhere, so that with increasing demand it was decided to make available a sufficiently detailed description of the outfit to enable anyone with a working knowledge of electricity to construct a satisfactory piece of apparatus.

DETAILS OF CONSTRUCTION

The parts listed with the wiring diagram, figure 1, can be obtained for a cost not exceeding twenty-five dollars from any wholesale radio supply house. Since the directions to follow are based on the use of these materials it is desirable to purchase the parts as specified.

The diagram of the upper side of the panel in figure 2 and the photograph of the lower side in Plate I show the position of the instruments. It is convenient to make a templet from stiff paper showing the exact position of all parts and drill all the holes through both the panel and the copper shield at the same time. The large holes can be cut with a circle cutter or sawed out. A number of holes in the copper shield will need to be made larger to prevent grounding of the instruments. All binding posts except Nos. 10 and 12 must be carefully insulated. The two variable resistors are insulated by a washer of fiber or mica. The wire leading to the center of the double throw switch must be very well insulated from the shield to

¹ Goodhue, Schwarte, and Fulmer, Iowa State College J. Sci. 7:111 (1933).

² Ellis and Kiehl, Rev. Sci. Inst. 4:131 (1933).

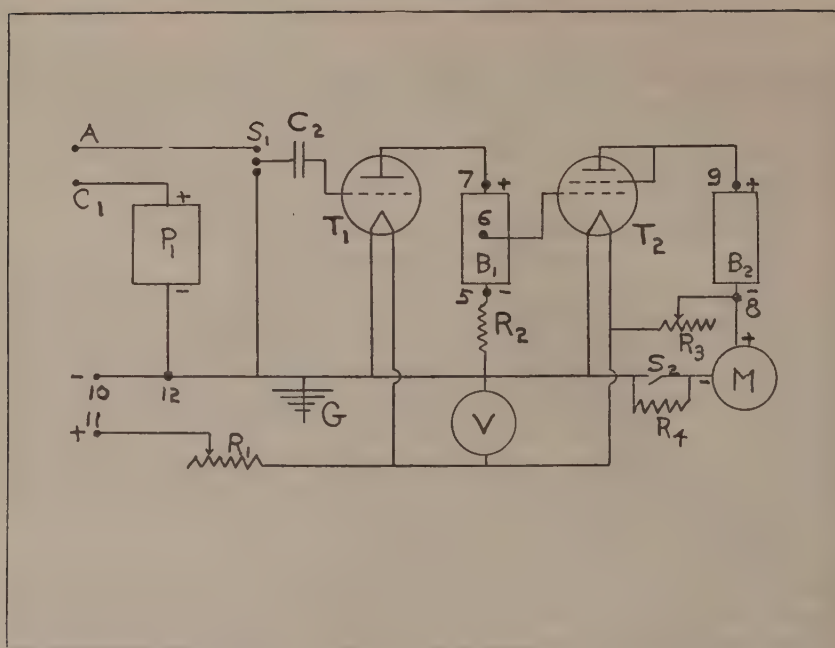


Fig. 1—Wiring diagram and list of materials: A—To center terminal of double throw knife switch—B₁, B₂—45 volt "B" batteries with tap at 22½ volts; C₁—To calomel half cell; C₂—0.001 mfd. mica condenser moulded in bakelite; G—Ground; M—Type 321 Triplett DC milliammeter range 0-1 ma.; P₁—Student type potentiometer; R₁—10 ohm rheostat with off position; R₂—150,000 ohm fixed resistance 1 watt capacity—R₃—2000 ohm wirebound nontapered radio potentiometer; R₄—5000 ohm fixed resistance 1 watt capacity; S₁—Electrometer switch; S₂—Toggle or jack switch single throw; T₁—No. 30 radio vacuum tube; T₂—No. 33 radio vacuum tube; V—Small inexpensive DC voltmeter 0-3 volts; Nos. 8, 9, etc.—Binding posts coded Fig. 2.

Bakelite panel 7 x 12 x 3/16 inches; 1 base mounting 4-prong socket; 1 base mounting 5-prong socket; 1 small double throw knife switch base about 1 x 2½ inches; 6 insulated binding posts marked plus; 4 marked minus; 2 pointer knobs; 100 feet No. 18 solid push back wire with lacquered outer braid; 1 doz. soldering lugs; Rosin core solder; 1 doz. round head cadmium plated machine screws 6/32 x 1 inch with nuts; 1 piece of sheet copper 6 x 11 inches; Copper gauze to line cabinet; Enough ¾ inch lumber to make a box with outside measurements of 7 x 12 and 6 inches deep.

prevent electrical leaks. This double throw switch could be replaced by another well insulated switch designed to be mounted under the panel and operated by a knob on the top. This would be desirable in laboratories where the instrument is subject to corrosion. The tube sockets are mounted the thickness of two nuts above the shield and the negative of the four-prong socket is bent down and soldered to the copper, as is the H nearest the K on the five-prong socket. The four-prong socket is mounted near the electrometer switch.

The sensitivity of the set depends on the quality of the mica condenser used. It should either be one sealed in oil or moulded in bakelite.

The condenser should be tested for leakage by applying 200 volts DC across the terminals and measuring the amount of current that leaks through by a sensitive galvanometer. A condenser is satisfactory if the leakage under these conditions is not over 0.01 microamperes. Condensers may also be tested by determining the rate at which they lose their charge. To eliminate body capacity effects, it is necessary to attach this condenser directly to the grid connection of the four-prong tube socket or to a lead not more than one inch long.

The proper working of the electrometer switch is so important that exact details will be given for its construction. The switch, figure 3, is mounted on pyrex glass rods A clamped in a block of wood B which serves as a base for mounting on the bakelite panel P. On the end of one of these glass rods a brass collar C holds a piece of No. 18 platinum wire. The wire is soldered to the collar in such a manner that it extends out

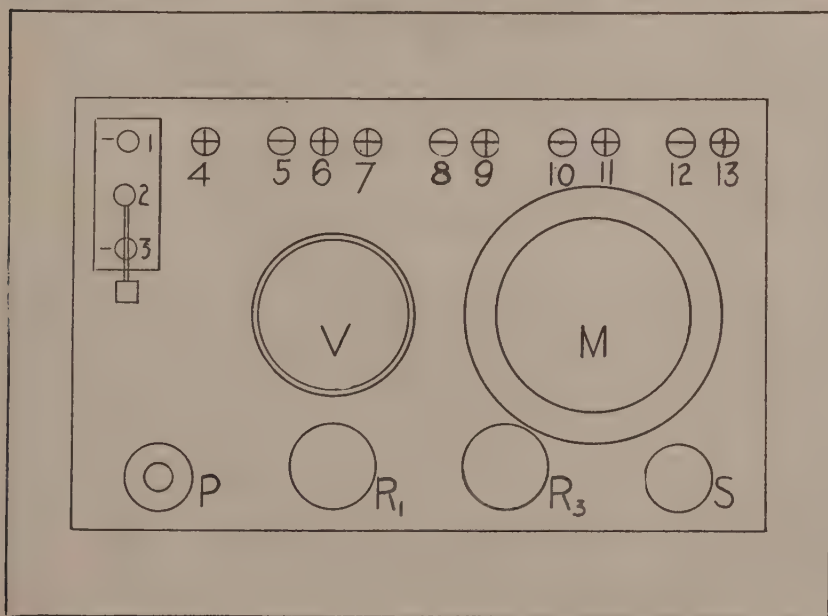


Fig. 2—External binding post connections: No. 1—To glass electrode; 3—To negative of Weston standard cell; 4—To calomel half cell and positive of Weston cell; 5—To negative of 1st 45 volt "B" battery; 6—To 22½ volt tap of 1st "B" battery; 7—To positive 45 volt tap of 1st "B" battery; 8—To negative of 2nd "B" battery; 9—To positive 45 volts of 2nd "B" battery; 10—To negative of two dry cells in series (3 volts); 11—To positive of two dry cells; 12—To negative of potentiometer and to ground; 13—To positive of potentiometer.

Internal binding post connections: No. 2—To terminal C of the electrometer switch; 4—To post No. 13; 5—To 150,000 ohm fixed resistance; 6—To control grid of 33 tube; 7—To plate of 30 tube; 8—To positive of milliammeter and to 2000 ohm potentiometer; 9—To plate and screen grid of 33 tube; 10—To copper shield; 11—To ten ohm rheostat; 12—To copper shield; V—Small volt meter; M—Milliammeter used as indicator; P—Push button used as tapping key; R₁—Filament control rheostat; R₃—2000 ohm potentiometer to control milliammeter; S—Switch with resistance to protect milliammeter.

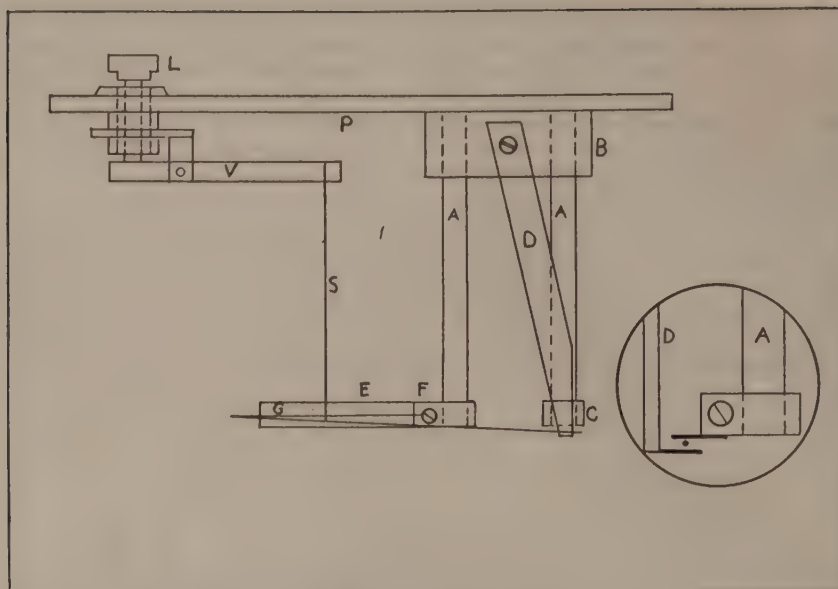


Fig. 3. Electrometer switch

three-eighths inch in the direction of the metal strip D and forms a right angle with the glass rod. The other rod holds a brass strip E to which is fastened a piece of No. 18 piano wire. The piano wire is held by the screw which clamps the brass strip at F and is also soldered at G. A 180° bend is made in the piano wire in the direction of the other glass rod. The end is tipped with three-eighths inch of No. 18 platinum wire, which crosses the first piece of platinum wire at right angles. The metal strip D attached by the bolt that holds the wood block together holds a piece of No. 18 platinum wire at the end below and directly parallel to the piece held by the brass collar C. The switch now consists of three points, two parallel to each other and a third working between and meeting them at right angles. The insert figure 3 shows an enlarged side view of the points. The tension of the piano wire is adjusted so that the contact presses firmly against the point leading to the ground. When the switch is operated the piano wire is pulled up, making a connection with C and the wire leading to binding post No. 2. The clearance between the points must be very small. This distance can be adjusted by swinging the metal strip D until all are in contact and then moving it back until a faint click is heard as the switch is operated. This metal strip must be very rigid (about three-eighths by one-eighth inch metal) in order to hold the point in the proper position.

The sleeve for the push button I is made from a small valve stem from an automobile tire. The hole is drilled out so that the three-sixteenths inch brass rod holding the button will move freely. The lever V and support are of brass strong enough to operate the switch. The end of the lever is connected to a point about one inch from the bend in the piano wire by a piece of silk fish line S and lacquered in position. The points of the switch should operate about twenty times faster than the push button.

After mounting, the switch is connected as follows: The point held by the collar C is connected by a lead to the center pole of the double throw knife switch. This wire must be well insulated from the ground to prevent electrical leaks. The metal bar D is grounded to the shield. The metal strip E is attached by a lead to the mica condenser, which is in turn connected to the grid of the first tube by a very short lead.

After all instruments are in place the set is ready to wire. The order usually followed is to connect the filament circuits first, then the grid and plate circuits afterwards. The binding posts and instruments are indicated by the same symbol in both figure 1 and figure 2. The explanation following figure 2 indicates clearly how each binding post should be connected. In figure 1 the copper shield takes the place of the negative wire from binding post No. 10 to the toggle switch S_2 and all wires leading into this one between these points are grounded to the shield. It may be necessary to use a little zinc chloride as a flux when soldering to the copper shield, but if this is done the parts where it is used must be thoroughly washed with water. Only rosin core solder should be used for all other connections. Each soldered joint should be tested by pulling on the wire in question. Binding posts Nos. 4 and 13 are connected by a wire under the panel. These posts are not absolutely necessary, but they tend to simplify the external wiring when the set is connected to the potentiometer.

TESTING THE SET

With the wiring now complete the set is ready to test. The tubes are placed in their sockets and the panel is placed on the box, allowing the tubes to hang up-side-down inside. First connect binding posts Nos. 10 and 11 with two dry cells in series and turn the rheostat slowly from its off position until the voltmeter reads 2 volts. If the filament circuit is connected properly the hand of the voltmeter should come up slowly to two volts as the knob of the rheostat is turned. If a tube is burned out the hand will jump almost instantly to three volts. When the filament current is turned on, the hand of the milliammeter should go down to the left. This is due to the compensating current which will balance the current flowing in the plate circuit of the second tube as soon as the "B" batteries are connected. While making these tests the toggle switch under the milliammeter should be turned off to avoid damaging the instrument. Connect the "B" batteries, the first to binding posts Nos. 5, 6, and 7, and the second to posts Nos. 8 and 9. Now turn on the filament circuit and adjust the milliammeter on the scale by turning the knob of the potentiometer. If the hand does not move in the direction the knob is turned the wires can

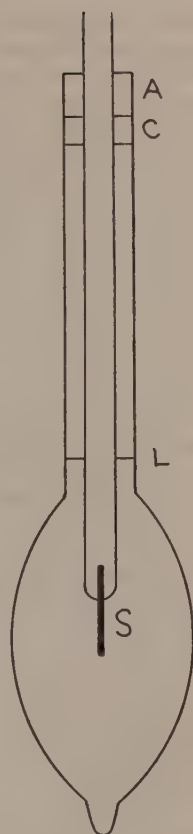


Fig. 4—Glass electrode. A—Sealing wax; C—Cork ring; L—Level of 0.2 N hydrochloric acid; S—Silver chloride electrode.

easily be shifted to make it do so. Since not all radio tubes are alike, the resistance in the plate circuit of the first tube may have to be changed in order that the hand of the milliammeter can be brought on the scale. In any case, this resistance should be such that about half of the 2000 ohm potentiometer is used when the hand is on the scale. This allows for fluctuations in the batteries as they become weaker.

The next step is to adjust the electrometer switch so that there is no extraneous movement of the hand of the milliammeter when the switch is operated. Connect the center pole of the knife switch with the negative binding post leading to the student potentiometer. This is a better ground than the negative "A" terminal, since the current flowing there may make a small difference in potential. Now, there should be no movement of the indicator when the button is pushed down. The button should be held firmly and moved down and up with a rapid, even motion. A slight click of the electrometer switch should be heard. If the indicator still moves, the points of the switch may have too much clearance or the tension on the piano wire is not enough. If the extraneous movement can not be taken out by adjusting the switch, another No. 30 vacuum tube should be tried. With some tubes it is impossible to eliminate this movement while with others no difficulty is encountered.

The outfit is now ready to be hooked up to the potentiometer. One of the student type potentiometers manufactured by Leeds and Northrup is excellent for the purpose. If other types are used all open parts of the potentiometer circuit must be closed. If tapping keys are built into the potentiometer one of these must be held down or a short circuiting switch provided. If separate binding posts are provided for a galvanometer they must be connected by a wire. The photograph (Pl. II) shows the complete outfit with a standard cell in a suitable box. The large box is lined with copper gauze and connected to the negative terminal of the potentiometer. It is sometimes necessary to ground all of the shielding.

The amount of shielding and the ground for one of these sets depends upon the resistance of the cell to be measured. If the cell has little or no resistance it can be measured with no ground or shielding, but as the resistance increases the amount of shielding must increase and a good ground is necessary. Usually a water pipe is good enough, but if its potential is not constant a separate ground will have to be used.

In measuring cells such as those containing a glass electrode it is sometimes observed that the hand of the indicator is unstable. Even at perfect balance the needle creeps to the right when the button is pressed down. This difficulty is encountered in very damp weather and in laboratories where alternating currents are too close to the outfit. This can easily be remedied by placing a grounded shield around the cell being measured and properly grounding the rest of the outfit. Usually a three-sided shield is sufficient around the glass electrode and calomel half cell if any is required at all. Sometimes placing the electrodes on a piece of grounded sheet metal will be satisfactory.

A MODIFIED BULB TYPE GLASS ELECTRODE

The type of glass electrode shown in figure 4 is suitable for student and industrial laboratory use where a rugged electrode is required. The electrodes are made from 10 mm. Corning glass No. .015. With a little

practice anyone can make these electrodes. It is not necessary to have them especially thin, since the outfit just described will measure across a very high resistance.

To blow the electrodes, heat a narrow part near the middle of a piece of the special glass about eight inches long and pull it out to a spindle. By breaking the spindle near the middle there is glass enough for two electrodes. Next, stopper the large open end of one of the tubes. Heat the other end of the glass just above the shoulder of the spindle until it is soft and quickly blow the elongated bulb by blowing, pulling, and turning all at the same time. This operation is done with the tube in a vertical position so that air currents cool the bulb evenly. The spindle is next sealed off and the electrode is complete.

The electrodes are filled a little way above the bulb with 0.2 N hydrochloric acid and a silver chloride electrode prepared by the method of Brown³ is inserted and sealed at the top with sealing wax. A rubber stopper around the top of the electrode is used for handling and should fit a test tube containing dilute hydrochloric acid, where the electrodes are stored while not in use. If the electrodes are broken the silver chloride electrode on the inside can easily be melted out and introduced into a new glass electrode. If time does not permit the electrodes do not need to be soaked over night in hydrochloric acid as recommended by some authors. The voltage obtained may be a little different with the fresh electrode, but with the method to be described later all of these differences are cancelled out.

DETERMINATION OF pH WITH GLASS ELECTRODE

After standardizing the potentiometer against the standard cell, the knife switch is thrown to the other position to allow an unknown potential to be measured. Unless the emf. to be measured is known to within 0.2 volts, the toggle switch protecting the milliammeter should be turned off until approximate adjustment is made.

Using a saturated calomel half cell for reference, the emf. is first determined when the glass electrode is immersed in 0.05 molar potassium acid phthalate. The voltage most often obtained is about 0.150 volts. Measure the voltage with the glass electrode immersed in the unknown solution. The difference in voltage E is used in the following equation to calculate the pH of the solution.

$$\text{pH} = 3.97 \pm \frac{E}{0.0591}$$

The sign in the equation is positive when the voltage obtained with the unknown solution is higher than that obtained with the potassium acid phthalate solution. A set of tables can be calculated from the equation or they can be copied from an article by Youden and Dobrosky⁴.

This method has proved to be the most convenient method to convert glass electrode potentials to pH readings. All changes in potential of the glass electrode cancel out, as do the fluctuations in voltage of the reference cell due to changes in temperature. Tables calculated from the equa-

³Brown, J. Am. Chem. Soc., 56:646 (1934).

⁴Youden and Dobrosky, Contrib. Boyce Thompson Inst., 3:347 (1931).

tion using the factor 0.0591 for 25°C. are accurate enough for most purposes between the temperatures of 20 and 30°C. A simple calculation shows that the tables are not over 0.03 pH in error at pH 10. Probably the glass electrode is more in error at the upper limits of the table than the amount due to temperature changes.

PLATE I

An electron tube nul-instrument for use with the glass electrode and a description of a rugged type of glass electrode.

ABOVE—Photograph of the under side of the panel.

BELOW—The complete outfit ready for operation.

PLATE I



A COMPARATIVE STUDY OF SEVERAL STRAINS OF THE SO-CALLED RADIOBACTER¹

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The bacteria belonging to the species commonly referred to as *Bacillus radiobacter* are widely distributed in soils. They are found closely associated with both the symbiotic and the non-symbiotic nitrogen-fixing bacteria. They occur frequently, therefore, in large numbers in close proximity to the roots of leguminous plants and in soils supporting a vigorous flora of *Azotobacter*. It has been found that additions to the soil of such materials as oat straw stimulate nitrate assimilation at first and later nitrogen fixation, and *Azotobacter* and *radiobacter* occur in large numbers. The frequency of the occurrence of *radiobacter* in soils and the variability of strains as emphasized by many investigators make it desirable to study the characteristics of a number of strains of this organism.

HISTORICAL

In a study of nitrogen-fixation in soils, Beijerinck and van Delden (2) observed an organism growing in close association with *Azotobacter chroococcum*. The organism was described as being a motile or non-motile rod which did not produce endospores. The colonies were small, slimy, soft or viscous. Gelatin was not liquefied but old gelatin cultures became iridescent. Acid and gas were never produced on sugar, but the culture solutions were always faintly alkaline. Organic acid salts such as acetate, malate, citrate, and propionate were rapidly oxidized to carbon dioxide and water. Vigorous foaming was observed in 0.02 per cent KNO_2 bouillon which was assumed to be a result of denitrification. The non-motile rods were somewhat larger than the motile rods, often curved and frequently united into a star-like mass, which was caused by the manner of cell division. However, this property of ray-formation was not considered a characteristic feature of the organism. The optimum temperature was 25-27°C. The organism was named *Bacillus radiobacter*.

Löhnis (5) isolated an organism from the soil which, in spite of some differences, was acknowledged by Beijerinck to be identical with *B. radiobacter* and thus there was an early indication of the variation in cultural and morphological characteristics of the organism. Denitrification in KNO_2 bouillon was not observed in Löhnis' work. He suggested a close relationship of the organism to the nodule bacteria.

Riker et al (7) described *B. radiobacter* as being actively motile by means of a single polar flagellum.

Löhnis and Hansen (6) stated that "numerous varieties of *B. radiobacter* could be isolated from every soil."

¹ Journal Paper No. J260 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 224.

It is, therefore, suggested from the literature that several species, or at least different strains of the organism, exist.

EXPERIMENTAL

The object of this investigation was to study the morphological, cultural and physiological characteristics of a number of strains of *B. radiobacter*.

Seven strains of the organism were secured from various investigators², one strain was secured from the American Type Culture Collection and 16 strains were isolated from soil by the method suggested by Smith (8) for the isolation and identification of *B. radiobacter*. Dilution plates were poured on glycerol-nitrate agar and colonies transferred to nitrate agar slants until the organism was obtained in pure culture.

Several staining procedures were employed with varying degrees of success. A modification of Liefson's (4) method for demonstrating flagella and capsules was the most successful of the several methods used for this purpose. The flagella stains were made by making a suspension of the growth from young agar slants in a small quantity of sterile distilled water. After 20 to 30 minutes, a loopful of this suspension was allowed to run down a clean, warm slide. The mordant and stain were each allowed to remain on the slide 10 minutes.

MORPHOLOGY

All organisms were gram-negative and appeared as rods 0.3 to 0.5 microns wide and 0.9 to 2.3 microns long. They occurred singly and in pairs; however, they occurred singly and in chains in hanging-drop preparations. They were all stained by the ordinary aniline dyes. All strains were actively motile. One to four flagella attached at the side or near the end of the cell appeared in most cultures, but two and three flagella were common. Some cells showed peripheral staining with the flagella attached to one end while others appeared as deeply stained rods with a distinct capsule. Many small oval-shaped cells appeared with a single flagellum. The same variations appeared in all strains, figure 1.

CULTURAL AND BIOCHEMICAL FEATURES

Glycerol-nitrate-agar colonies: The colonies were raised, glistening, smooth, with white center and transparent edge. In strain 36 the cells of some colonies appeared to be united in a star-like mass which gave the appearance of rays extending from the center of the colony. This is the only instance ray-formation was observed. Colonies of strain 36 had a tendency to flatten out after 4 to 6 days, while colonies of strain 4718 were spherical or drop-like and had no tendency to spread out flat. Both types of colonies were found in all other strains. The white center often appeared as concentric rings, especially in strains of 4, 38, 130, 3, 1000, 22 and 131.

²Strains 1 and 4, courtesy Dr. Roy Hansen, Urbana, Illinois; 14, Dr. J. K. Wilson, Ithaca, N. Y.; 36 and 188, Mr. N. R. Smith, Bureau of Chemistry and Soils, Washington, D. C.; 1000 and 1018, Dr. I. L. Baldwin, University of Wisconsin, Madison, Wis.; 4718, American Type Culture Collection, Chicago, Ill.; 130, 131, 20, 21, 3, 6, 7, 8, 9, 10, 11, 120, 121, 13, 22, and 38, isolated from various Iowa soils.



Fig. 1. *B. radiobacter*, 24-hour old cultures at 28° C., showing flagella.

Glycerol-nitrate-agar slants: Growth was abundant, raised, smooth, glistening and transparent in young cultures, whereas old cultures became granular and opalescent. Old cultures of strain 36 became dry and opaque. Old cultures of strain 188 were opaque and gelatinous. Old cultures of all strains were brownish and granular.

Potato: Scanty and brown.

Broth: Cloudy. Sediment and a pellicle were formed after 7 days.

Milk: Brown, except with strain 36, which was variable, and strain 22, which produced no change in color after 3 weeks.

Litmus-lactose agar: Reduced, except by strain 36.

Gelatin: Not liquefied.

Starch: Not hydrolyzed.

Indol: Negative.

Acetyl-methyl-carbinol: Negative.

Hydrogen sulfide: Negative.

Nitrate: Disappeared (reduced or assimilated).

Reaction: Abundant growth on glycerol-nitrate agar was observed over range of pH 4.5 to 9.5.

FERMENTATIONS

Cultures were inoculated in triplicate on agar slants to determine the fermentation characteristics. The medium contained:

| | | |
|--|------|------|
| K_2HPO_4 | 1.0 | gram |
| $NaNO_3$ | 1.0 | " |
| Organic carbon compound ^a | 1.0 | " |
| Agar | 10.0 | " |
| Brom-thymol blue, 0.5 per cent alcoholic solution | 5.0 | c.c. |
| Distilled water | 1000 | c.c. |
| Reaction adjusted to pH = 6.9 to 7.0 | | |

The medium was made according to the procedure suggested by Baldwin and Fred (1). The cultures were incubated at 27-28°C. and readings made after 3, 6, 17 and 28 days.

Good growth was made on all of the carbon compounds used in the fermentation studies. Cultures of strains 1000 and 1018 sometimes produced a slightly acid reaction in sodium citrate and potassium tartrate after 3 days, but the reaction was usually alkaline after 6 or 17 days. A slight to strongly acid reaction was developed by some strains, but the reaction produced by many strains was strongly basic to brom-thymol blue. Where the reaction change was only slightly acid to brom-thymol blue, the reaction of duplicate cultures was often basic. The test was repeated and similar results were obtained.

Quantitative tests on the utilization of carbon compounds shows these organisms to be very efficient in the utilization of dextrose. One

^a The following compounds were used as sources of carbon: dextrose, d-mannose, levulose, galactose, arabinose, xylose, rhamnose, maltose, lactose, sucrose, trehalose, raffinose, starch, dextrin, mannitol, glycerol, inulin, salicin, dulcitol, sodium formate, sodium citrate, sodium malate, sodium succinate, sodium lactate and potassium tartrate.

TABLE 1. *The pH of media after five days incubation at 28°C.*

| Cul- ture | Arabi- nose | Rham- nose | Dex- trose | Levu- lose | Galac- tose | Suc- rose | Lac- tose | Starch | Dex- trin | Inu- lin | Sali- cin | Man- nitol |
|--------------|----------------|---------------|---------------|---------------|----------------|--------------|--------------|--------|--------------|-------------|--------------|---------------|
| Control | 6.47 | 6.46 | 6.69 | 6.01 | 6.49 | 6.69 | 6.58 | 6.87 | 6.68 | 6.40 | 6.72 | 6.68 |
| 1 | 7.13 | 7.18 | 7.85 | 6.90 | 7.12 | 7.02 | 7.32 | 7.30 | 7.52 | 7.66 | 7.29 | 7.57 |
| 4 | 7.20 | 7.20 | 7.47 | 7.07 | 7.02 | 7.11 | 7.24 | 7.19 | 7.60 | 7.63 | 7.15 | 7.47 |
| 14 | 6.43 | 6.43 | 7.58 | 6.51 | 6.68 | 6.38 | 6.59 | 6.88 | 6.98 | 7.03 | 6.74 | 6.80 |
| 36 | 6.65 | 6.13 | 6.97 | 6.36 | 6.71 | 6.30 | 6.62 | 6.76 | 6.91 | 7.28 | 6.55 | 6.66 |
| 188 | 5.96 | 6.43 | 7.77 | 5.34 | 6.56 | 5.43 | 6.88 | 6.64 | 7.40 | 6.99 | 6.70 | 5.75 |
| 1000 | 6.46 | 6.63 | 7.02 | 6.11 | 6.51 | 6.44 | 5.70 | 6.74 | 6.83 | 7.62 | 7.21 | 6.49 |
| 1018 | 7.17 | 7.05 | 7.69 | 6.81 | 6.99 | 7.01 | 7.03 | 7.02 | 7.02 | 7.59 | 7.23 | 7.44 |
| 4718 | 7.16 | 7.34 | 7.64 | 7.01 | 7.00 | 7.01 | 7.12 | 7.25 | 7.33 | 7.60 | 7.20 | 7.50 |
| 20 | 7.11 | 7.02 | 7.79 | 6.95 | 6.70 | 6.93 | 7.17 | 7.25 | 7.25 | 7.19 | 6.58 | 7.34 |
| 21 | 7.15 | 6.48 | 7.80 | 7.05 | 6.73 | 6.92 | 6.89 | 7.36 | 7.29 | 7.43 | 6.56 | 7.31 |
| 3 | 6.58 | 7.05 | 7.06 | 6.34 | 6.73 | 6.84 | 7.05 | 7.04 | 6.88 | 7.00 | 6.76 | 6.80 |
| 6 | 6.57 | 6.66 | 6.82 | 6.55 | 6.71 | 6.46 | 6.34 | 6.99 | 6.88 | 7.36 | 6.83 | 6.56 |
| 7 | 6.82 | 6.63 | 7.80 | 6.56 | 6.84 | 6.66 | 7.08 | 7.32 | 7.30 | 7.65 | 6.90 | 7.23 |
| 8 | 6.78 | 6.48 | 7.86 | 6.85 | 6.51 | 6.82 | 7.16 | 7.25 | 7.32 | 7.64 | 6.90 | 7.27 |
| 9 | 6.69 | 6.58 | 6.92 | 6.33 | 6.60 | 6.60 | 5.99 | 6.83 | 6.96 | 7.45 | 6.80 | 6.62 |
| 10 | 6.98 | 7.33 | 7.70 | 6.96 | 6.86 | 6.76 | 7.16 | 7.12 | 7.08 | 7.58 | 7.08 | 7.37 |
| 11 | 6.52 | 6.66 | 7.08 | 6.40 | 6.56 | 6.53 | 6.02 | 6.62 | 6.62 | 7.62 | 6.90 | 6.63 |
| 120 | 6.97 | 6.16 | 7.95 | 6.93 | 6.79 | 6.93 | 7.09 | 7.29 | 7.14 | 7.54 | 7.02 | 7.33 |
| 121 | 7.07 | 7.14 | 7.80 | 6.85 | 7.00 | 6.68 | 6.57 | 7.22 | 7.23 | 7.60 | 6.96 | 7.34 |
| 13 | 7.01 | 7.11 | 6.96 | 6.92 | 7.07 | 6.97 | 6.94 | 6.97 | 7.04 | 7.62 | 6.92 | 7.35 |
| 130 | 6.71 | 7.15 | 7.66 | 6.61 | 6.61 | 6.56 | 7.27 | 7.10 | 7.46 | 7.71 | 7.01 | 7.06 |
| 131 | 6.87 | 7.10 | 7.60 | 6.85 | 6.67 | 6.72 | 7.09 | 7.00 | 7.12 | 7.66 | 7.12 | 7.20 |
| 22 | 6.71 | 7.17 | 6.93 | 6.84 | 6.69 | 6.19 | 6.84 | 6.87 | 7.24 | 7.53 | 6.94 | 7.12 |
| 38 | 6.64 | 7.35 | 7.32 | 6.76 | 6.82 | 6.50 | 7.08 | 7.01 | 7.40 | 7.62 | 6.96 | 7.23 |

hundred c.c. of a medium containing 40.0 mgm. of carbon as dextrose were inoculated and incubated for 5 days at 28°C. Carbon dioxide evolution was determined after 3, 4 and 5 days. The numbers of organisms and the amount of dextrose remaining in the solution were also determined at the 3, 4 and 5-day periods. The data showed that after 5 days there were 0.69 mgm. of carbon as dextrose left in the culture, 35.61 mgm. carbon as carbon dioxide had been evolved, leaving 3.7 mgm. carbon unaccounted for. There was an increase of 80 billion organisms in the culture during the 5 days. From these data it is apparent that there was not much dextrose left for acid production.

Another fermentation test was made similar to the first one described, except that the organisms were grown in a solution culture medium and the reaction was determined electrometrically. Potassium di-hydrogen phosphate was used instead of the di-potassium phosphate and the amount of KH_2PO_4 was also decreased in order to decrease the buffer capacity of the medium. The medium employed contained:

| | |
|------------------------------|-----------|
| KH_2PO_4 | 0.1 gram |
| MgSO_4 | 0.1 " |
| NaNO_3 | 1.0 " |
| Carbon compound ⁴ | 1.0 " |
| Distilled water | 1.0 liter |

Triplicate tubes of each medium were inoculated, incubated 5 days at 28°C. and the pH determinations made. The data obtained are presented in table 1 and represent the averages of closely agreeing duplicates.

TABLE 2. *The pH change in media by strains of B. radiobacter producing acid reaction*

| Organ- ism | Carbon compound | | | | | | | |
|---------------|-----------------|---------------|---------------|--------------|--------------|--------|--------------|---------------|
| | Arabi- nose | Rham- nose | Levu- lose | Suc- rose | Lac- tose | Starch | Salic- in | Man- nitol |
| 188 | 0.51 | — | 0.67 | 1.26 | — | 0.23 | — | 0.93 |
| 36 | — | 0.33 | — | 0.39 | — | 0.11 | 0.17 | — |
| 120 | — | 0.30 | — | — | — | — | — | — |
| 1000 | — | — | — | 0.25 | 0.88 | 0.13 | — | 0.19 |
| 11 | — | — | — | 0.16 | 0.56 | 0.25 | — | — |
| 20 | — | — | — | — | — | — | 0.14 | — |
| 21 | — | — | — | — | — | — | 0.16 | — |
| 6 | — | — | — | 0.23 | 0.24 | — | — | 0.12 |
| 22 | — | — | — | 0.50 | — | — | — | — |
| 14 | — | — | — | 0.31 | — | — | — | — |
| 7 | — | — | — | 0.03 | — | — | — | — |
| 9 | — | — | — | 0.09 | 0.59 | — | — | — |
| 121 | — | — | — | 0.01 | 0.01 | — | — | — |
| 130 | — | — | — | 0.13 | — | — | — | — |
| 38 | — | — | — | 0.19 | — | — | — | — |

⁴The carbon compounds used were arabinose, rhamnose, dextrose, levulose, galactose, sucrose, lactose, starch, dextrin, inulin, salicin and mannitol.

The data show that in most cases the medium was more basic after inoculation and incubation than the controls. In a few cases the medium in the inoculated tubes was slightly more acid than the controls. The organisms producing acid and the pH change for the different carbon compounds are shown in table 2.

The data show that the pH changes on the acid side of the control are relatively few in number and only slight, except for strain 188, where there was a pH change of 1.26 for sucrose, 0.51 for arabinose, 0.67 for levulose, 0.23 for starch and 0.93 for mannitol.

Another test was made to determine the reaction changes in dextrose and sucrose media of the same composition as that used in the preceding experiment except that $(\text{NH}_4)_2\text{SO}_4$ and NH_4NO_3 were used as sources

TABLE 3. *Effect of nitrogen source on pH change in dextrose and sucrose media by B. radiobacter*

| Organism | Dextrose | | Sucrose | |
|----------|------------------------------|--------------------------|------------------------------|--------------------------|
| | $(\text{NH}_4)_2\text{SO}_4$ | NH_4NO_3 | $(\text{NH}_4)_2\text{SO}_4$ | NH_4NO_3 |
| Control | 5.23 | 5.07 | 4.82 | 4.98 |
| 188 | 4.51 | 4.53 | 4.82 | 4.98 |
| 4718 | 5.00 | 5.31 | 5.82 | 7.23 |

of nitrogen. Triplicate tubes of the different media were inoculated with strains of 188 and 4718 and incubated at 28°C. for 5 days. The pH of the inoculated and control cultures were determined electrometrically as in the preceding experiment. The data obtained are presented in table 3.

Strain 188 produced a slight acidity in the dextrose media over that of the control, but the change was not quite as large in the NH_4NO_3 medium as in the $(\text{NH}_4)_2\text{SO}_4$ medium. Good growth was obtained with strain 188 in the sucrose media, but no change in reaction was produced. Strain 4718 produced a slight acidity in the dextrose $(\text{NH}_4)_2\text{SO}_4$ medium, but the reaction of the dextrose NH_4NO_3 medium was made slightly more basic than the control. Strain 4718 produced no change in the reaction of the sucrose $(\text{NH}_4)_2\text{SO}_4$ medium, but the reaction of the sucrose NH_4NO_3 medium was made strongly basic relative to that of the control.

DISCUSSION

Considerable variation in the size and shape occurred among the cells of a single strain, but the cells of the various strains varied similarly. Certain parts of the cell stained intensely while other parts of the cell did not stain at all. In some cells the entire contents of the cell stained evenly and in others one or two bodies stained deeply or the center and cell wall only took the stain. However, no consistent differences between strains were observed and no differentiation microscopically could be made.

There was considerable variation in the appearance of colonies of single cultures, but the types of colonies were common to all strains. On glycerol-nitrate-agar slants old cultures of strain 36 appeared dry and rough, young cultures of strain 4718 appeared somewhat more granular than other strains. Different shades of brown in young milk cultures were

often obtained, but after 3 weeks all strains, except No. 22, were dark reddish-brown and produced a small amount of serum which was also dark reddish-brown in color. Growth in broth was similar in all strains. Since the variations noted were common to all strains, no differentiation of the various strains by cultural features could be made.

Good growth was made on all of the carbon compounds used in the fermentation studies. The media were usually made alkaline to bromthymol blue, but a slightly acid reaction was sometimes developed, which later changed to alkaline. Quantitative studies on the utilization of dextrose by strain 4718 indicated a low acid production and a rather complete fermentation of the sugar. The assimilation of the nitrate-nitrogen or the reduction of the nitrate produced an alkaline reaction when NaNO_3 or NH_4NO_3 were used as the source of nitrogen. Even in a medium of low buffer capacity with $(\text{NH}_4)_2\text{SO}_4$ as the source of nitrogen, the use of indicators to denote fermentation by *B. radiobacter* is, as Conn and Darrow (3) have pointed out, a procedure which requires caution.

When NaNO_3 was used as the source of nitrogen, 12 of the 24 strains produced a slight to strongly acid reaction in sucrose, but all strains increased the pH of the dextrose medium. The sucrose was undoubtedly less completely oxidized than the dextrose. The pH of the media was increased over that of the control by all strains in dextrose, galactose, dextrin and inulin; and in levulose by all strains, except strain 188. No considerable acid production was evidenced by any of the strains, except possibly that of strain 188, and no differentiation of the various strains on the basis of fermentation reactions could be made.

The results of these studies seem to indicate that these strains are all of one species of bacteria. The variations which occur in the morphological, cultural, and physiological characteristics, while possibly greater than in certain other species of bacteria, are not great enough to differentiate the strains into groups and the inference that several species are represented seems unwarranted.

SUMMARY AND CONCLUSIONS

Eight strains of the so-called radiobacter were secured from various laboratories in the United States and 16 were isolated from Iowa soils. Studies on the morphological, cultural, and physiological characteristics were made. No differences great enough to differentiate species were obtained and it was concluded that all cultures represent a single species.

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A DESIGN FOR TESTING TECHNIQUE IN CODLING MOTH SPRAY EXPERIMENTS¹

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The proper estimation of codling moth (*Carpocapsa pomonella* L.) field populations as a means of evaluating spraying experiments has always presented a serious obstacle to be overcome in the testing of spray materials. A survey of the literature discloses the rather startling fact that among the many workers experimenting along these lines there is little or no agreement in their approach to this rather essential phase of the work. These differences in technique indicate that some of the conclusions reached may be based on an unnecessary redundancy of data, or perhaps still more commonly, on insufficient or improperly analyzed data.

Theoretically, one should be able to lay out a carefully planned series of plots, and by reducing and eliminating variables, and applying statistical analysis to a complete set of plot and tree samples and subsamples, arrive at (1) valid estimates of treatment effect, (2) an estimate of experimental error, and (3) adequate tests of experimental technique.

With these objectives in mind such an attempt was made during the summer of 1934. On an apparently homogeneous block of 96 Ben Davis trees in an orchard at Mitchellville, Iowa, four tests, A, B, C and D, were quadruplicated in 16 six-tree plots in the familiar Latin square arrangement. All trees received a calyx spray of lead arsenate and five cover sprays of their respective test schedules during the season. Test A was a check plot and was sprayed throughout the season with lead arsenate at three pounds to 100 gallons. Test B received calcium arsenate at the same rate with one pound of ferrous sulfate and two pounds of hydrated lime added to each 100 gallons to act as a buffer to prevent arsenical burning. Three pounds of manganese arsenate with one quart of fish oil as an adhesive were used to each 100 gallons on test C. For the first three cover sprays Test D was sprayed with lead arsenate as in Test A, but the fourth and fifth cover sprays consisted of one per cent mineral oil emulsion and nicotine sulfate at the rate of one pint per 100 gallons.

At harvest the total crop was counted on each of three trees per plot. These trees were selected from the six trees on the basis of uniformity in crop size and general conformation to average appearance. It seemed to the authors that the number of stings, number of worms per fruit, and the percentage infestation of dropped fruit, would not only be directly proportional to the percentage of wormy fruit at harvest, but would also tend to make unnecessarily more complicated an already involved situation. The actual record, therefore, was made of numbers of wormy fruits in successive lots of ten apples. This method has several advantages from

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the standpoint of facilitating statistical analyses. First, it makes possible the selection of any number of subsamples of any desired size, limited only by the size of the total crop of that particular tree. Second, since the order of counting the successive lots of 10 apples was preserved, it became possible by selecting random samples of 10 fruits or by using lots consisting of several hundred successively counted fruits, to arrive at an idea of the importance of random selection of tree subsamples. It should be explained that in examining the harvest crop, the fruit was left in boxes under the previously marked trees. The examining crew then began counting box by box at the end of the row, continuing until all the fruit had been examined.

Following is a sample record of one tree as preserved for future analysis.

TABLE 1. *Total count at harvest on sample tree, Oct. 16-19, 1934*

| Plot A—Row 3—Tree 2 | | | |
|--|------------------------|----------------------|--|
| Clark Orchard, Mitchellville, Iowa | | | |
| Number of wormy apples in a sample of 10 | | | |
| 4,5,3,4,2,4,5,4,5,3, | 6,4,2,3,4,5,4,2,6,6, | 5,3,5,6,3,3,2,2,5,2, | |
| 3,1,3,4,6,6,3,3,5,1, | 2,4,5,5,4,4,4,2,4,7, | 4,3,2,2,7,6,4,5,2,2, | |
| 5,7,4,4,4,3,5,1,2,4, | 2,3,3,4,4,2,1,3,3,2, | 6,5,3,3,0,3,4,4,5,3, | |
| 3,5,4,6,2,3,5,4,3,4, | 3,3,5,7,3,4,5,9,5,7, | 4,2,3,5,6,7,4,3,4,6, | |
| 4,3,5,5,3,5,3,4,4,7, | 4,3,2,4,3,4,5,7,4,3, | 5,3, | |
| Total wormy—556 | | | |
| Total fruit—1420 | Percentage wormy—39.15 | 5 bushels | |

A brief survey of these figures shows several things. The 142 lots of 10 apples contain from none to nine wormy fruits. Lots of 50 apples are from 22 per cent to 60 per cent wormy, whereas, lots of 100 vary from 27 per cent to 51 per cent wormy. This sequence of decreasing ranges follows roughly the law of normal distribution.

In making an analysis of variance it is desirable that each datum be on the same basis. It is not convenient, then, to use the actual number of wormy apples on each tree in the analysis, since the trees yielded different sizes of crops. If one desires to use actual numbers of wormy fruit one should select samples of uniform size from each tree. If, on the other hand, the complete crop is to be evaluated, the percentage of wormy fruit may be used. The results of three analyses of variance will be presented in this paper. The first analysis (table 3) is the one which yields the most significant information and is based upon 30 randomly selected lots of ten apples from each count tree. The second analysis (table 4) is based on 30 consecutive lots of ten apples from each count tree. The third analysis (table 5) is based on the percentage of wormy fruit in the total crop of each count tree and therefore could not include tree subsamples.

Involved in the first analysis (based on 30 randomly selected lots of ten apples from each tree) were 48 trees arranged in 16 three-tree plots. These 16 plots were arranged in a Latin square of four rows and four ranks. The four tests, A, B, C and D, were assigned to the 16 plots in such a way that a test occurred only once in each row and rank. Each test con-

sisted of 12 trees quadruplicated in three-tree plots, making a total of 360 lots of 10 apples for each test and a grand total of 1440 samples for the entire analysis. In table 2 the thirty subsamples for each tree have been summed and entered in the 48 cells of the accompanying table.

TABLE 2. *Number of wormy apples in 30 random lots of 10 apples in each of 3 trees in each of 4 tests*

| | Row 1 | Row 2 | Row 3 | Row 4 | Total |
|--------------------|-----------|-----------|-----------|-----------|-------|
| Rank 1 | C | B | A | D | |
| | 156 | 188 | 124 | 101 | |
| | 92 | 169 | 182 | 109 | |
| | 119 | 138 | 153 | 115 | |
| | <hr/> 367 | <hr/> 495 | <hr/> 459 | <hr/> 325 | 1646 |
| Rank 2 | A | D | B | C | |
| | 167 | 63 | 143 | 161 | |
| | 166 | 61 | 142 | 165 | |
| | 150 | 63 | 136 | 140 | |
| | <hr/> 483 | <hr/> 187 | <hr/> 421 | <hr/> 466 | 1557 |
| Rank 3 | B | C | D | A | |
| | 94 | 152 | 114 | 122 | |
| | 137 | 141 | 116 | 127 | |
| | 95 | 113 | 36 | 119 | |
| | <hr/> 326 | <hr/> 406 | <hr/> 266 | <hr/> 368 | 1366 |
| Rank 4 | D | A | C | B | |
| | 68 | 165 | 101 | 199 | |
| | 136 | 166 | 109 | 216 | |
| | 164 | 160 | 136 | 206 | |
| | <hr/> 368 | <hr/> 491 | <hr/> 346 | <hr/> 621 | 1826 |
| Total | 1544 | 1579 | 1492 | 1780 | 6395 |
| | Test A | Test B | Test C | Test D | |
| Test totals | 1801 | 1863 | 1585 | 1146 | |
| Test means | 50.03% | 51.75% | 44.03% | 31.83% | |
| (percentage wormy) | | | | | |

The analysis of variance was calculated according to the methods outlined by Fisher² and Snedecor³.

In analyzing the variance between the different plots, it becomes apparent that not all the variation is due to differences in rank or row or even between tests. The mean square of this residue is denoted as experimental error. In order for differences to be significant, their mean squares must be several times larger than the experimental error, the exact ratio (F) depending on the number of observations or replications (degrees of

² Fisher, R. A. Statistical methods for research workers. Oliver & Boyd, Edinburgh. Fourth edition (1933).

³ Snedecor, George W. Calculation and interpretation of Analysis of Variance and Covariance. Collegiate Press, Inc., Ames, Iowa. (1934).

TABLE 3. *Analysis of variance of number of wormy fruits in 30 random plots of ten apples each*

| Type of analysis | Source of variation | Degrees of freedom | Sum of squares | Mean square | Standard deviation |
|------------------------------------|--------------------------------|--------------------|----------------|-------------|--------------------|
| Analysis of variance between plots | Total | 1439 | 6845.7 | 4.758 | 2.18 |
| | Between tests | 3 | 881.2 | 293.7 | |
| | Between rows | 3 | 136.0 | 45.33 | |
| | Between ranks | 3 | 308.7 | 102.9 | |
| | Remainder, error | 6 | 475.9 | 79.33 | |
| Analysis of variance within plots | Between trees in the same plot | 32 | 2388.9 | 74.63 | |
| | Within trees | 1392 | 2655.0 | 1.907 | 1.38 |
| | Total within plots | 1424 | 5043.9 | 3.543 | 1.88 |

freedom). Such tests of significance showed that, in this particular experiment at least, position in row or rank contributed no more variation than would be expected from random variation, indicating that the 12 trees per test might have been chosen in solid unreplicated blocks. Unless the tests had been replicated, however, we would have had no assurance of this apparent fact. The F value between tests is 3.7 (293.7/79.33), which is very close to the five per cent point of significance. This apparent lack of significant differences between tests is very interesting when we recall that the maximum difference, that between test B and test D, was 51.75 per cent minus 31.83 per cent or nearly 20 units. We must conclude, however, that because of the large experimental error and small number of degrees of freedom we cannot be entirely certain that this difference of 20 units is not a freak of variation instead of a real difference in treatments. It will be shown later in this paper that with a more refined analysis significant differences do exist in effects of tests, even though they cannot be shown as differences in actual percentage of wormy fruit.

TABLE 4. *Analysis of variance of number of wormy fruits in 30 consecutive lots of 10 apples each*

| Type of analysis | Source of variation | Degrees of freedom | Sum of squares | Mean square | Standard deviation |
|------------------------------------|--------------------------------|--------------------|----------------|-------------|--------------------|
| Analysis of variance between plots | Total | 1439 | 7162.7 | 4.978 | 2.23 |
| | Between tests | 3 | 837.0 | 279.0 | |
| | Between rows | 3 | 337.1 | 112.4 | |
| | Between ranks | 3 | 501.6 | 167.2 | |
| | Remainder, error | 6 | 643.7 | 107.3 | |
| Analysis of variance within plots | Between trees in the same plot | 32 | 3692.3 | 115.4 | |
| | Within trees | 1392 | 1151.0 | .827 | .91 |
| | Total within plots | 1424 | 4843.3 | 3.401 | 1.85 |

Table 4 shows an analysis of the same trees used in table 2, but is based on subsamples selected consecutively instead of at random. The chief difference between this and the previous analysis is the larger mean square attributed to experimental error in table 4. The reason for the greater error of the consecutive selections becomes apparent if one understands the method generally used in picking and examining the fruit. It is customary in most sections for the pickers to fill their bags at one place on a tree, possibly from the ground, a ladder, or on the inside of the tree. A picking bag may sometimes hold a bushel of fruit, and of course would be emptied into one box or basket. If, then, the examiner counted 300 apples from two baskets, both of which had been filled with fruit from the ground or the inside of a tree, he would probably find comparatively few wormy fruit. If, at the next tree, 300 fruits were counted, all of which were picked from the top of the tree where there was an unusually high percentage of wormy fruit, his counts would have shown a large difference in worminess between the two trees, whereas, if random samples had been examined they would probably have shown correctly that the two trees were about equally wormy. As one would expect, then, table 4 shows a much less significant difference between tests. Perhaps it should be pointed out that the latter method is inefficient not because the experimental error is large, for under special cases the error might actually be smaller than if random samples had been used. The real objection to consecutive samples is that they do not measure the population as accurately as random samples.

TABLE 5. *Analysis of variance of percentage of wormy fruit in 48 trees*

| Source of variation | Degrees of freedom | Sum of Squares | Mean squares |
|---------------------|--------------------|----------------|--------------|
| Total | 47 | 9601.9 | 204.3 |
| Between tests | 3 | 3552.1 | 1184.0 |
| Between rows | 3 | 569.4 | 189.7 |
| Between ranks | 3 | 1089.1 | 362.8 |
| Remainder | 6 | 1998.0 | 332.8 |
| Within plots | 32 | 2393.3 | 74.8 |

The third analysis (table 5) is based on the percentage of wormy fruit in the total crop of each count tree and therefore could not include tree subsamples. Since with this type of analysis we have no way of obtaining any idea of the variation within the tree it is the least valuable of the three from the standpoint of studying technique, although it will show test differences as well as, if not slightly better than, the use of random tree subsamples. The doubtful advantage of the added numbers, however, is clearly not worth the increased labor involved in obtaining them.

Table 6 is a tabulation showing the effects of the four spray schedules evaluated in four different ways. The F value is directly proportional to the significance of the difference between test means.

Returning to table 3 one notices from the standard deviations that a certain amount of variation is present within each tree, or within each group of 30 subsamples. The standard deviation between subsamples of a

TABLE 6. *Mean percentage of wormy fruit based on different types of data*

| Type of data from each tree | F | Average percentage wormy | | | |
|---------------------------------------|------|--------------------------|----|----|----|
| | | A | B | C | D |
| 30 random lots of 10 fruits each | 3.7 | 50 | 52 | 44 | 32 |
| 30 consecutive lots of 10 fruits each | 2.6 | 50 | 52 | 45 | 33 |
| Complete counts (unweighted means*) | 3.6 | 52 | 53 | 45 | 31 |
| Complete counts (weighted means) | | 52 | 42 | 48 | 29 |

* Unweighted means were calculated by averaging the twelve tree-percentages (although based on crops of varying size). Weighted means were calculated by dividing total wormy fruit on the twelve trees by the total crop on the same trees.

single tree is 1.38; between the 90 subsamples of a plot it increases to 1.88, and between the total of 1440 subsamples it is 2.18 wormy fruits per 10 apples. The highest standard deviation (2.18) is made up of several types of variation. By analysis of variance procedure, the row, rank and test differences were isolated, but of the remaining unexplained variation, the fractions due to within tree variation, between tree variation, and between plot variation have not been adequately separated. The problem then becomes one of deriving these ratios by additional analysis.

The variation unaccounted for by test, row and rank mean squares is 79.33. This variation is made up of tree and sample variations as well as unaccountable plot variation. One may assume,

A = Plot variation
 B = Tree variation
 C = Sample variation

Since there are 30 samples per tree and three trees per plot, we must multiply B or the tree variation by 30, and the A or plot variation by 3×30 , to make them comparable to the sample variation. Then,

$$79.33 = 90A + 30B + C$$

But B or the tree variation is influenced by the variation within trees. So, if $C = 1.907$, the mean square of the variation between trees, but within plots,

$$74.63 = 30B + 1.907$$

$$B = 2.424$$

$$\text{Then, } 79.33 = 90A + (30 \times 2.424) + 1.907$$

And we have, finally,

Plot variation (A) = 0.031
 Tree variation (B) = 2.424
 Sample variation (C) = 1.907

It should be borne in mind that these values of A, B and C are not actual differences, but mathematical functions facilitating theoretical comparisons.

From our values of A, B and C we conclude (A) that comparatively insignificant benefit would be gained by greater plot replication; (B) that we can reduce experimental error most efficiently by increasing the number of trees in the test, and (C) somewhat less by using more than thirty 10-apple samples from each tree.

An attempt has been made in this experiment to control or record all the factors possible that might affect worminess in a certain tree. It has long been supposed that a tree with a small crop will tend toward a heavy percentage of wormy fruit. If this hypothesis were true those tests applied to trees with small crops might appear unusually and erroneously ineffective. Analysis of covariance not only furnishes a means of testing the validity of this hypothesis, but also of evaluating treatment effects with this correlation considered.

TABLE 7. *Number of apples/10 and percentage wormy on 48 trees in 4 tests of 12 trees each*

| Test | A | | B | | C | | D | |
|-----------------|----------------------------|------------------|--|------------------|--|------------------|--|------------------|
| | Wormy | No. apples 10 | Wormy | No. apples 10 | Wormy | No. apples 10 | Wormy | No. apples 10 |
| 12 trees | 64 | 175 | 74 | 246 | 59 | 82 | 52 | 153 |
| | 61 | 213 | 73 | 156 | 58 | 60 | 45 | 146 |
| | 57 | 292 | 71 | 124 | 56 | 111 | 38 | 112 |
| | 57 | 208 | 61 | 95 | 53 | 215 | 37 | 256 |
| | 56 | 106 | 58 | 105 | 50 | 139 | 37 | 176 |
| | 54 | 219 | 51 | 151 | 46 | 149 | 36 | 115 |
| | 54 | 39 | 48 | 43 | 43 | 200 | 34 | 75 |
| | 53 | 56 | 47 | 70 | 42 | 241 | 24 | 372 |
| | 48 | 261 | 46 | 45 | 40 | 177 | 22 | 256 |
| | 42 | 176 | 42 | 311 | 38 | 231 | 22 | 177 |
| | 39 | 142 | 33 | 84 | 30 | 257 | 20 | 276 |
| | 38 | 105 | 31 | 69 | 27 | 399 | 14 | 208 |
| Sum | 623 | 1,992 | 635 | 1,499 | 542 | 2,261 | 381 | 2,322 |
| Sum of squares | 33,125 | 398,662 | 35,975 | 260,391 | 25,692 | 518,413 | 13,503 | 527,320 |
| Sum of products | 104,977 | | 83,379 | | 92,796 | | 68,342 | |
| Grand totals | Sum of pctg. wormy = 2,181 | | Sum of squares of pctg. wormy = 108,295 | | Sum of squares of pctg. wormy = 108,295 | | Sum of squares of pctg. wormy = 108,295 | |
| | Sum of no. apples = 8,074 | | Sum of squares of no. apples = 1,704,786 | | Sum of squares of no. apples = 1,704,786 | | Sum of squares of no. apples = 1,704,786 | |
| | | | Sum of products = 349,489 | | | | | |

A superficial examination of the above table shows that although there are many exceptions, the general tendency is for the percentage of wormy fruits per tree to increase as the number of apples decreases.

By reference to table 8 we find that the correlation between totals is barely significant and that between tests not significant, but that the correlation within tests (in which we are particularly interested) is highly so. Trees with small crops actually do tend to be unusually wormy, although the correlation between worminess and crop size is small when the tests are considered as units.

TABLE 8. *Analysis of variance and covariance, percentage wormy and number of fruits/10 on 48 trees in 4 tests of 12 trees each*

| Source of variation | Degrees of freedom | Mean square Pctg. wormy | Mean square number fruits/10 | Covariance | | |
|---------------------|--------------------|-------------------------|------------------------------|-----------------|--------|-------------|
| | | | | Sum of products | r | Re-gression |
| Total | 47 | 195.8 | 7524.9 | —17373.4 | —0.309 | —0.050 |
| Between tests | 3 | 1141.3 | 11708.4 | — 8278.2 | —0.265 | —0.235 |
| Within tests | 44 | 131.2 | 7080.6 | — 9095.2 | —0.605 | —0.029 |

It has been shown by this analysis of covariance that crop size has a very definite effect on percentage of wormy fruit. If one examines the figures in table 7 it becomes apparent that for some unknown reason, those tests which yielded the smallest percentage of wormy fruit actually were applied to plots bearing the heaviest crops. The problem now resolves itself into the necessity of evaluating the different tests with the crop size taken into consideration. An analysis of the variance of the sums of squares of the errors of estimate of worminess from crop size gives us a method of doing this. It can be shown that the sum of squares of the errors of estimate are equal to $C - (B^2/A)$ where $C = \sum \text{wormy}^2$, $B = \sum \text{crop}^2$ and $B = \sum (\text{wormy} \times \text{crop})$. The various sums of squares can be read or calculated from table 8.

TABLE 9. *Analysis of variance of sum of squares of errors of estimate of percentage of wormy fruit from number of fruit on tree*

| Source of variation | Degrees of freedom | Sum of squares of errors of estimate | Mean square |
|---------------------|--------------------|--------------------------------------|-------------|
| Total | 46 | 8349.2 | 181.5 |
| Between tests | 2 | 1473.0 | 736.5 |
| Within tests | 43 | 5507.3 | 128.1 |
| Difference | 1 | 1368.9 | 1368.9 |

The analysis in table 9 tells us that since the mean square error of estimate between tests is 5.74 (or F) times as large as its experimental error (in this case, within test mean square), we conclude that when size of crop is considered, there actually is a significant difference between the effects of the various spray schedules. In this particular experiment, then, the four tests did not have a significantly different worm infestation, but did differ significantly in the amount of reduction of worm population from that expected from the four different sizes of crops. A second conclusion demonstrated by table 9 is that, due to the large difference between the total sum of squares and that attributed to sums of squares between and within test, it becomes apparent that the dependence of worminess upon crop size is significantly different between tests from the regression within tests.

Approximate tests of individual differences show that Test D (receiving oil-nicotine in second brood sprays) is significantly better than A and B, and questionably better than C.

SUMMARY

An experimental design is described whose fundamental purpose was to enable the authors to test the technique of sampling and general plot design.

The total crop was counted, worminess being recorded from successive samples of 10 fruits each. From these series of figures three sets of samples were selected and several analyses of variance were made to test the efficiency of the various methods of sampling. It was concluded that random selection of 300 apples gave an adequate picture of tree infestation.

A negative correlation ($-.605$) was shown to exist between crop size and percentage of wormy apples on individual trees. The correlation between crop size and worminess on 12-tree tests was significantly smaller ($-.265$).

Although no significant differences were directly demonstrable between the four tests, when the crop size was considered analysis of variance of the errors of estimate showed significant differences to exist, the test receiving oil-nicotine in the second brood sprays being definitely better than calcium or lead arsenate, and questionably better than manganese arsenate.

OBSERVATIONS ON YEASTS CAUSING GAS IN SWEETENED CONDENSED MILK¹

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The formation of gas is one of the more common defects of sweetened condensed milk. Although Rogers and Clemmer (6) found that an organism belonging to the genus *Aerobacter* was the cause of gas formation in a sample of sweetened condensed milk examined by them, it appears from the general studies on this defect that yeasts are usually responsible.

While most of the yeasts reported to have been isolated from gassy sweetened condensed milk have not been described in detail, they show a number of common characters and apparently fall into two types on a morphologic basis. A recent outbreak of gas formation, in which yeasts were involved, afforded an opportunity to compare the causative organism or organisms with the descriptions of various yeasts isolated from gassy sweetened condensed milk. The data obtained are reported herein.

HISTORICAL

Pethybridge (5) isolated two types of yeasts from gassy sweetened condensed milk put up at an Irish condensery. Each type formed gas in sweetened condensed milk and in saturated sucrose bouillon. The predominant yeast was oval and measured about $0.9 \times 3.6 \mu$. It produced gas from dextrose and sucrose but not from lactose and did not grow well in the absence of fermentable sugar. Inoculated tubes of sweetened condensed milk showed gas formation after about 19 days at 37° C. or 24 days at 20° C. The other yeast isolated by Pethybridge was spherical with a diameter of 5.5μ . It fermented sucrose but not lactose and grew fairly well on sugar free media. Gas was produced in sweetened condensed milk in about 20 days at 37° C.

Hunziker (3) secured yeasts from gassy sweetened condensed milk; these grew well in supersaturated sucrose whey bouillon and produced gas when inoculated into cans of normal milk. The yeasts were of the same general type and the cells were rather large and oval in form.

In a study of an outbreak of gas formation in sweetened condensed milk, Hammer (1) isolated a yeast which he considered responsible for the defect. The organism grew in saturated sucrose bouillon and produced gas in cans of sweetened condensed milk in from 2 to 6 days at 37° C. although with one brand high in milk solids gas was not produced in some instances. The organism was oval in form and somewhat wider and shorter than the oval organism reported by Pethybridge (5). Hammer described the organism in considerable detail and named it *Torula lactis-condensi*.

¹ Journal Paper No. J265 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 119.

Knudsen (4) isolated two types of yeasts from gassy sweetened condensed milk. Both types produced gas in sweetened condensed milk and fermented dextrose, levulose and sucrose but not galactose, maltose and lactose. From Danish sweetened condensed milk only a spherical type was secured, while from Dutch sweetened condensed milk both a spherical and an oval type were isolated. The spherical type measured 5 to 6 μ in diameter, developed gas in a sucrose substrate between 7° and 35° C., showed good surface growth on gelatin with slow liquefaction, and produced alcohol slowly in 30 per cent dextrose bouillon. The oval type measured 2 to 3 by 4 μ , developed gas in a sucrose substrate between 14° and 35° C., showed no surface growth on gelatin and produced alcohol rapidly in 30 per cent dextrose broth. Knudsen assumed that his yeasts were identical with those found by Pethybridge (5). Unstained cells of the oval type were larger than the cells in stained preparations; the size of the stained cells corresponded closely to that reported for *T. lactis-condensi* (1).

Two types of yeasts capable of producing gas in milk containing 70 per cent sucrose and in sweetened condensed milk were isolated by Hiscox (2). One, a spherical type, measured 5 to 6 μ in diameter and developed a heavy white growth on beerwort agar, while the other, an oval type, measured 1.5 to 2.7 μ x 3 to 4.7 μ and developed poorly on beerwort agar. The biochemical characteristics of the two types were practically identical. Both fermented dextrose, levulose and sucrose but not maltose and lactose while fermentation of galactose was questionable. Milk with as high as 70 per cent sucrose added, showed vigorous gas formation followed by coagulation. Inoculated cans of sweetened condensed milk held at 22° C. bulged after 10 to 17 days with the spherical type and after 4 days with the oval type. Stained cells of the oval type were about the same size as that reported for *T. lactis-condensi* (1), but unstained cells were larger. The two types were compared with the organisms isolated by Knudsen (4) and found to be identical.

HISTORY OF THE OUTBREAK

The outbreak of gas formation involved the output of a plant, in a neighboring state, that marketed sweetened condensed milk in barrels. The development of gas occurred more or less frequently in the milk packed during several summer months. Most of the defective lots of milk did not develop gas until some time after they had left the plant. In some cases the heads of the barrels were bulged while in other cases they were not; evidently the extent to which the organisms had developed varied, and this was presumably related to the temperature and time of holding and to the degree of the contamination. When barrels with bulged heads were opened gas rushed out and often milk was thrown for some distance.

The spoiled milk had a yeasty odor, and yeasts were found in it in rather large numbers when it was examined microscopically. The yeasts were easily isolated when the milk was smeared on the surface of agar containing considerable sucrose.

EXPERIMENTAL

Isolation of cultures. Samples from six lots of the gassy sweetened condensed milk were studied. The causative organisms were isolated by

plating the milk on sucrose (30 per cent) agar, or by smearing it on the surface of this medium, and picking colonies into dextrose or sucrose bouillon after incubating the plates at 21° C. Two types of yeasts capable of producing gas when inoculated into sweetened condensed milk were secured. An oval type was isolated from all six samples, while a spherical type was found in only one. The sample yielding both types of yeasts also contained still other yeasts that produced gas in sucrose bouillon, but which failed to produce gas in sweetened condensed milk.

Identity of the organisms. A detailed study of the morphologic, cultural, and biochemical characteristics of both the oval and the spherical types of yeasts was made. The oval type was identified as *T. lactis-condensi*, although it differed somewhat in size from that originally reported for this organism (1). The spherical type appeared to be similar to, if not identical with, the unnamed spherical yeasts isolated by Pethybridge (5), Knudsen (4), and Hiscox (2).

DESCRIPTION OF *TORULA LACTIS-CONDENSI* (Fig. 1)

The cultures of *T. lactis-condensi* studied included 11 cultures from the outbreak and 6 additional cultures isolated from two samples of gassy sweetened condensed milk recently submitted to the Iowa Agricultural Experiment Station from different sources. The description of *T. lactis-condensi*, as enlarged by studies on the cultures recently isolated, is as follows:

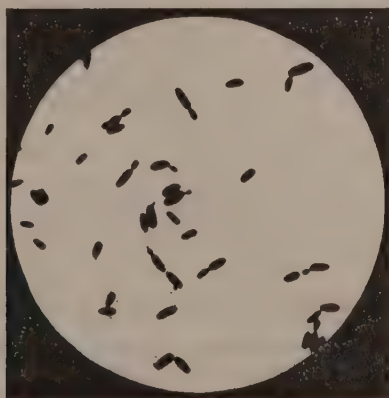


Fig. 1. *Torula lactis-condensi*

Morphology (at 21° C.)

Form and size. Cells in stained preparation from 48 hour potato dextrose agar slope cultures were typically oval, the size of the majority ranging from 1.0 to 1.5 μ in width and from 2.0 to 3.5 μ in length; in aqueous suspensions of the living cells the majority ranged from 1.6 to 2.4 μ in width and from 3.2 to 4.8 μ in length.

Arrangement. Cells from young potato dextrose agar cultures were usually single or with one bud attached.

Staining reactions. Cells in young cultures were usually gram positive while in old cultures many of the cells were gram negative.

Spore formation. Spores could not be demonstrated in cultures grown on potato dextrose or malt agar for from one day to several months, or in cultures grown on gypsum blocks for various periods.

Cultural Characteristics (at 21° C.)

Agar slope. After 24 hours on sucrose, malt, or potato dextrose agar growth was evident as small, round, smooth, slightly raised, translucent, shiny, white, non-viscid colonies. On further incubation the colonies fused and the growth became more opaque. Growth was scant on standard agar or whey agar. Little difference was noted in the rate or extent of growth on agars containing percentages of sucrose ranging from 1 to 30²; most of the cultures showed some growth on saturated sucrose agar.

Agar colony. After 48 hours on potato dextrose agar the colonies were small, round, smooth, slightly raised, shiny, white and non-viscid, translucent at first and increasing in opacity with age.

Sucrose (30 per cent) agar stab. Vigorous gas formation occurred and resulted in an extensive breaking up of the medium.

Gelatin stab. Growth was slight, both with and without 0.3 per cent yeast extract (Difco) added but extended along the entire depth of the puncture; the gelatin was not liquefied.

Potato. No growth was evident.

Bouillons. Growth was slight in the absence of fermentable materials. Where growth occurred the medium became turbid in from 24 to 48 hours and then cleared, leaving a heavy sediment.

Dunham's solution. No growth was evident.

Uschinsky's solution. No growth was evident.

Litmus milk. No change occurred.

Sucrose milk. Active gas formation and coagulation (in 2 to 3 days) occurred in milk containing from 1 to 50 per cent sucrose; gas production and coagulation were slower with the higher concentrations than with the lower. Most of the cultures produced considerable gas in milk saturated with sucrose.

Biochemical Features (21° C.)

Fermenting power. In bouillons, acid and gas were produced in 2 to 4 days from dextrose, levulose, and sucrose; slight acid but no gas was produced in 4 to 10 days from raffinose and occasionally from maltose. Neither acid nor gas was produced from arabinose, rhamnose, galactose, lactose, inulin, dextrin, starch, glycerol, dulcitol, mannitol, sorbitol, adonitol, inositol, or salicin.

Oxygen relationship. The organism was facultative; it grew very well aerobically in the presence of a fermentable sugar.

Growth temperatures. On potato dextrose agar the organism did not grow at 7° or at 40° C.; it grew slowly at 15° C., rapidly between 20° and 35° C., and fairly well at 37° C.

²Throughout the description the concentrations of sucrose represent the percentage by weight in the finished media.

TORULA GLOBOSA SP. NOV.

The cultures of the spherical yeast studied included 1 culture from the outbreak and 2 cultures isolated from a sample of gassy sweetened condensed milk recently submitted to the Iowa Agricultural Experiment Station; both of the samples of milk that yielded this type also contained *T. lactis-condensi* and, according to colony development on plates and direct microscopic observation, the latter organism was present in greater numbers than the former. The spherical yeast resembled *T. lactis-condensi* in many respects, including gas production in sweetened condensed milk, staining reaction, inability to form spores, growth on gelatin and in litmus milk, fermenting powers, and oxygen relationships. It appears to be identical with the spherical yeasts isolated from gassy sweetened condensed milk by various investigators. The name *Torula globosa* is suggested for it. *T. globosa* differed from *T. lactis-condensi* in the following characters, all the determinations being made at 21° C.

Form and size. The majority of the cells in stained preparations from 48 hour potato dextrose agar slope cultures were spherical, although a few of the cells tended to be slightly oval; the diameter of the majority of the cells ranged from 2.5 to 4.0 μ . With aqueous suspensions of the living cells the diameter of the majority ranged from 4.4 to 6.8 μ .

Growth on agar slope. Growth on potato dextrose or malt agar was more abundant than with *T. lactis-condensi* and was opaque, creamy white, dull, and raised. There was considerable growth on standard agar and on whey agar.

Agar colony. After 48 hours the colonies on potato dextrose or malt agar were larger, more raised, dull, and opaque than those of *T. lactis-condensi*.

Potato. Growth was fairly abundant, creamy white, shiny, and non-viscid.

Bouillons. There was fair growth in the absence of a fermentable material.

Dunham's solution. Growth was fair.

Uschinsky's solution. Growth was slight.

Sucrose milk. In milk containing various percentages of sucrose, gas production and coagulation were somewhat slower than with *T. lactis-condensi*, especially with sucrose concentrations of 40 per cent or more. No gas was produced in saturated sucrose milk.

Growth temperatures. On potato dextrose agar the organism did not grow at 4° or 37° C.; it grew slowly at 7° C., rapidly between 15° and 30° C. and fairly well at 35° C.

GAS PRODUCTION BY *T. LACTIS-CONDENSI* AND *T. GLOBOSA* IN SKIM MILK CONTAINING SUCROSE

Skim milk containing various percentages of sucrose was prepared by adding the required amount of sucrose to skim milk (10 grams of sucrose to 90 grams of skim milk equalled 10 per cent sucrose, etc.), dissolving the sucrose by heating and dispensing in about 10 ml. quantities in test tubes; skim milk was saturated with sucrose by dissolving as much sucrose as possible by heating and then adding this mixture to tubes containing small amounts of sucrose. The sucrose milk was sterilized at 15 pounds for 20 minutes. After cooling, the tubes intended to

contain the milk saturated with sucrose showed many crystals while the tubes containing 70 per cent sucrose yielded large crystals of sucrose when seeded with a few small crystals.

T. lactis-condensi produced gas actively at 21° C. in the 10, 20, 30, 40, or 50 per cent sucrose milk in 2 days, and in the 60 per cent sucrose milk in 3 to 4 days; in the 70 per cent sucrose milk some of the cultures failed to produce gas while the others produced it in from 7 to 12 days, and in the saturated sucrose milk some of the cultures failed to produce gas but most of them produced it in from 5 to 20 days. The milk was coagulated when gas formation became evident or within a few days after, coagulation being slower than gas formation with sucrose concentrations of 50 per cent or more.

T. globosa produced gas rapidly at 21° C. in the 10, 20, or 30 per cent sucrose milk in 2 days, in the 40 or 50 per cent sucrose milk in 3 days, and in the 60 per cent sucrose milk in 5 days; no gas was produced in the 70 per cent or in the saturated sucrose milk. Coagulation of the milk was slower than with *T. lactis-condensi*, usually occurring 1 or 2 days after gas production was evident.

GAS PRODUCTION BY *T. LACTIS-CONDENSI* AND *T. GLOBOSA* IN SWEETENED CONDENSED MILK

When sweetened condensed milk was transferred aseptically from normal cans to sterile test tubes and inoculated with *T. lactis-condensi* or *T. globosa*, gas was regularly produced in from 12 to 17 days at 21° C.

Cans of normal sweetened condensed milk were inoculated by covering a small portion of one end of each can with concentrated HCl, flooding this area with solder, punching a hole in the center with a sterilized nail, and then adding a small portion of a young sucrose bouillon culture by means of a capillary pipette; the can was immediately resealed with solder and inverted several times to distribute the organisms through the material. Controls of punched and resealed or of unopened cans were incubated along with the inoculated cans.

Occasionally, for no evident reason, an inoculated can failed to develop gas, even with an extended holding period, but commonly gas production occurred. *T. lactis-condensi* usually produced gas in the inoculated cans at 21° C. and at 35° C. When gas production occurred the cans bulged in from 7 to 15 days, and some of the cans eventually ruptured, usually along a seam. *T. globosa* did not produce gas at 35° C. but produced it in from 12 to 15 days at 21° C.; the cans usually ruptured after an extended holding period.

With either *T. lactis-condensi* or *T. globosa*, sweetened condensed milk in test tubes was thickened rather firmly, while in cans the change in consistency seemed to be limited to the formation of lumps or a ring of thick milk at the surface.

DEGENERATION OF *T. LACTIS-CONDENSI*

A culture of *T. lactis-condensi* from the original isolation (1), that had been subcultured more or less regularly for about 17 years, was studied along with the cultures recently isolated. It had lost its ability to produce gas in sweetened condensed milk, and the cells were considerably larger and growth on artificial media more abundant than when it was first isolated.

SUMMARY AND DISCUSSION

Six samples of gassy sweetened condensed milk from a recent outbreak were studied; all the samples yielded an oval yeast while one of them also contained a spherical type. Two additional samples of gassy sweetened condensed milk that had been submitted for examination yielded the oval yeast while one of them also contained the spherical yeast. The oval type was identified as *Torula lactis-condensi*, the description of which was checked and enlarged. The name *Torula globosa* was suggested for the spherical type, which apparently is identical with the spherical yeasts isolated from gassy sweetened condensed milk by various investigators. The differences between *T. lactis-condensi* and *T. globosa* involve primarily morphology, extent of growth on solid media in the absence of a fermentable material and growth temperatures.

From the reports of previous investigations and the data herein presented it appears that gas formation in sweetened condensed milk is commonly due to *T. lactis-condensi* or to *T. lactis-condensi* and *T. globosa* growing together, and that when the two species are growing together *T. lactis-condensi* is the more numerous. However, in a sample of Danish milk that had blown, Knudsen (4) found only the spherical yeast.

The failure to secure gas production in certain instances when *T. lactis-condensi* or *T. globosa* was inoculated into a can of normal sweetened condensed milk may be due to various factors. A culture may lose its ability to grow in high concentrations of sugar and other materials; in this connection the failure of a culture of *T. lactis-condensi* from the original isolation to produce gas in sweetened condensed milk after an extended period of transfers is of interest. It appears also that in a viscous material, like sweetened condensed milk, the difficulty with which the organisms are distributed, either naturally or with shaking, may be a factor in limiting their growth.

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THE ALKALINE MERCERIZATION OF WOOL

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Cold concentrated alkali was observed by John Mercer in 1845 (12) to increase the dyeing property of wool, and in British Patent 13,296 of 1850 (14) he recommended an 18 per cent or less concentrated solution of sodium hydroxide at a temperature below 10° C. for this process. Kertesz (10), Knecht (11), Bucher (2), and Giesler (6) have recommended the use of concentrated alkali in printing pastes for wool. Schneider considered the dyeing property of wool depended on the concentration, time, and temperature of its alkaline pretreatment (19), especially the removal of sulfur (18).

Cold concentrated sodium hydroxide, 11 to 26 per cent, has been used to crêpe fabrics of cotton and wool as these concentrations do not appreciably shrink wool (German Patent 30,966 of 1884, 14, 17).

The enhancement of the luster of wool by cold concentrated sodium hydroxide has been described by Hesse (8) and German Patent 113,205 (14).

Buntrock (3) studied the effect of various concentrations of sodium hydroxide in 10 minutes at room temperature on the strength of wool and noted that 15 per cent sodium hydroxide was the most destructive and that the dry breaking strength was increased by 33 to 50 per cent sodium hydroxide, the 38 per cent producing a maximum increase of 34 per cent over that of the original wool. Washburn (21) and Matthews (12) repeated Buntrock's work and reported the sulfur content, 3.42 per cent, of a wool as reduced to 0.53 per cent by 38 per cent sodium hydroxide at 15° C. in five minutes. Speakman (20) found the elastic properties of single wool fibers unaffected after immersion in 38 per cent sodium hydroxide for five minutes at 19° C. and ascribed the increase in strength of wool yarn to surface gelatinization of the fibers. Jäger (9) has described 18 per cent sodium hydroxide as the most destructive to wool in 10 minutes at room temperature and an increase in the dry strength of the wool as the concentration of alkali is increased from 18 to 50 per cent.

Reported here are the changes in composition and mechanical failure of a plain-woven wool upon mercerization with 16, 29, 38, and 44 per cent sodium hydroxide for five minutes at 15° C. as measured by the weight, nitrogen, sulfur, dry and wet warp breaking strength and elongation at breaking load.

EXPERIMENTAL PROCEDURE

Preparation of wool. Approximately six grams of plain-woven undyed wool were boiled one hour in 100 volumes of distilled water, dried, extracted continuously with anhydrous ether eighteen hours in a modified Soxhlet extractor, and dried at 105° to 110° C. until successive weighings checked within a half milligram. Upon analysis this wool yielded 0.30 per cent of ash, no sulfates (13), and 0.21 per cent of sulfite-yielding

sulfur (7) as compared with 0.25 per cent obtained with scoured wool fiber.

Preparation of concentrated alkali. Several days after more than the calculated amount of sodium hydroxide was dissolved in boiled distilled water the solution was filtered, its specific gravity at 15° C. determined with a Westphal balance, and its dilution to the desired concentration effected with boiled distilled water.

Mercerization of wool. A six-gram sample or ten breaking strength specimens, wet and centrifuged, were immersed in 200 cc. of the alkali at $15 \pm 0.1^\circ$ C. and stirred constantly for five minutes. The wool was then stirred into successive liters of water until the rinse was not alkaline to phenolphthalein.

Analysis of wool. The wet breaking strength and elongation at breaking load of ten specimens were determined with a Scott Universal Tester and Autographic Recorder immediately after rinsing (1). The dry tests were made after drying and conditioning ten specimens for four or more hours at 65 ± 2 per cent R. H. and $70 \pm 2^\circ$ C.

The mercerized wool was dried at room temperature (15) and analyzed for nitrogen by the Kjeldahl-Gunning method (5). Each value is the average of four determinations corrected for the nitrogen of the reagents.

TABLE 1.

TABLE 1. *Effect of alkali in five minutes at 15° C. on the weight, nitrogen, sulfur, breaking strength, and elongation of wool*

| Sodium hydroxide | Residual Wool | | | | | | |
|------------------|-----------------|--------------------|--------|-------------------------------------|-------------------------------|-----------------------------|------------------------|
| | Weight | Nitrogen | Sulfur | Breaking strength of warp | | Elongation at breaking load | |
| | Percent- age | Percentage of wool | | Dry lbs. per in. of fabric | Wet percent- age of dry | Dry percent- age | Wet percent- age |
| | | | | | | | |
| 0 | 100.0 | 16.49 | 3.99 | 20 | 73 | 29 | 55 |
| 16 | 93.7 | 15.25 | 3.16 | 4 | — | 30 | — |
| 29 | 98.1 | 16.29 | 3.61 | 14 | 2 | 38 | — |
| 38 | 98.8 | 16.33 | 3.78 | 17 | 28 | 28 | 47 |
| 44 | 98.7 | 16.01 | 3.77 | 19 | 32 | 35 | 51 |

The wool was dried to constant weight at 105° to 110° C. before analysis for sulfur by the Benedict-Denis method (4). Six grams of wool were heated until dissolved in 100 cc. of a solution, one part nitric acid and two parts water, in a covered evaporating dish on a steam plate. After the addition of 100 cc. of Benedict-Denis reagent the mixture was evaporated to dryness and the residue heated to dull red for ten minutes, dissolved in 100 cc. of 10 per cent hydrochloric acid, and filtered. The filtrate was heated to boiling and the sulfur precipitated as barium sulfate by the addition of 100 cc. of 10 per cent solution of barium chloride. After fifteen hours the precipitate was filtered into a Gooch crucible (16), washed

free of chlorides, and ignited to constant weight. The sulfur in each case is the average of four determinations corrected for the sulfur of the reagents.

All weighing of bottles and crucibles was done with tares.

Table 1 shows the weight, nitrogen, sulfur, wet and dry breaking strength and elongation at breaking load of the wool before and after mercerization for five minutes at 15° C. in 16, 29, 38, and 44 per cent sodium hydroxide. Figure 1 expresses the weight, nitrogen, sulfur, and wet and dry breaking strength of the mercerized wool in terms of the original wool.

The changes in dry breaking strength are similar to those reported by Jäger (9) although the 5.3 per cent loss of sulfur by wool in 38 per cent sodium hydroxide for five minutes at 15° C. is in contrast to the 84.45 per cent loss reported by Washburn (21) and Matthews (12). The low wet breaking strength of mercerized wool argues against its use.

SUMMARY

1. The mercerization of plain-woven unstoved wool of 0.30 per cent ash and no sulfates, by 16, 29, 38, and 44 per cent sodium hydroxide for five minutes at 15° C. has been followed by weight, nitrogen, sulfur, dry and wet warp breaking strength and elongation at breaking load.

2. The loss in weight, nitrogen, and sulfur has been shown to decrease with an increase in concentration from 16 to 38 per cent sodium hydroxide and to be greater at 44 per cent sodium hydroxide. At 38 per cent sodium hydroxide the wool lost 1.2 per cent in weight, 1.0 per cent in nitrogen, and 5.3 per cent in sulfur.

3. The loss of wet and dry breaking strength was less, the greater the concentration of alkali. At 44 per cent sodium hydroxide the dry breaking strength was 95 per cent and the wet breaking strength but 40 per cent of the corresponding values for the original wool.

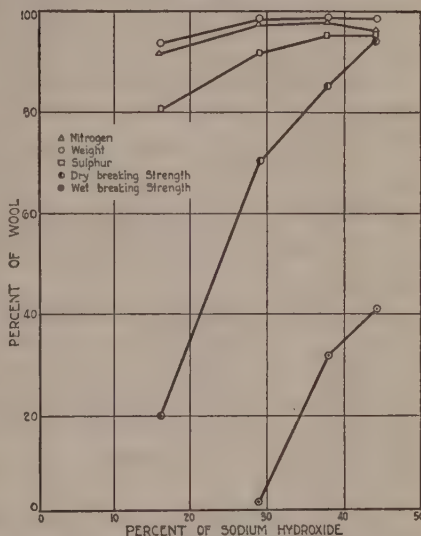


Fig. 1. Effect of alkali in five minutes at 15° C. on the weight, nitrogen, sulphur and breaking strength of wool.

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THE APPLICATION OF FURFURAL AND ITS DERIVATIVES TO THE MANUFACTURE OF PLASTICS¹

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To those uninitiated into the secrets of chemistry, there may seem little connection between the manufacture of plastics and the utilization of agricultural wastes. Furfural is the name of the chemical which forms the strongest link between the two.

Furfural has the unique record of changing in less than ten years from a laboratory curiosity to the cheapest aldehyde obtainable. The reason for this phenomenal development may be attributed to research, which had discovered many new uses for this interesting compound, and to the fact that furfural is derived from an inexpensive and inexhaustible supply of agricultural wastes.

It has been predicted that a system of furane chemistry will sometime be known, similar to the system of benzene chemistry we have now. A partial fulfillment of this prophesy has already taken place, as several furane derivatives analogous to aromatic compounds have been produced. The similarity between the benzene derivative and its furane twin suggests the possibility that furane compounds may be substituted for benzene derivatives in various technical applications. Such a substitution was made in the case of the preparation of the complex organic compounds used as accelerators in the vulcanization of rubber. As a result, accelerators derived from furfural are now the subject of patents. Although most of the commercial accelerators contain nitrogen, it was found that sulfur derivatives of furfural also possess the property of accelerating the vulcanization reaction. One of these derivatives, polythiofurfural, was prepared and tested by the writer for its value as an accelerator.

The preparation of polythiofurfural was based on the fact that furfural in dilute aqueous solution will react with hydrogen sulfide. As the gas is passed into the solution, polythiofurfural is formed in colloidal particles, which may be coagulated by the addition of a small amount of hydrochloric acid. A two to four per cent furfural solution was found to produce optimum results, and by passing the hydrogen sulfide in series through several absorption bottles, complete absorption of the gas was obtained.

Following the preparation of a quantity of the polysulfide, the compound was subjected to the general tests given an experimental accelerator. After a few trials, it became apparent from tensile strength tests that all the samples were being overcured. This indicated that the compound was of the ultra-accelerator type, rather than the less rapid group. Tests using various amounts of the accelerator disclosed the fact that a concentration of less than one per cent of the compound did not produce vulcanization, but amounts in excess of one per cent gave

¹ Original thesis submitted July, 1930.

promising results. Low temperatures cures (220° F.) resulted in high tensile strengths. Since the anodic deposition of rubber requires an ultra-accelerator in colloidal solution, furfural polysulfide was believed ideal, since it is prepared in the colloidal form. It was found, however, that the compound was not sufficiently active at the temperature of boiling water to effect a cure. The compound was found to be unsuitable for use in hard rubber formulae. Because of the low cost of the compound, it was found cheaper to use than some of the commercial accelerators, in spite of the necessity of using about five times as much accelerator. The tests conclusively proved that furfural polysulfide does have a remarkable accelerating effect on the vulcanization reaction.

One of the methods attempted for the purification of the polysulfide was its distillation in vacuo. At the close of the distillation, a crystalline product was removed from the distilling flask. Washing with petroleum ether removed the orange color, leaving white crystals which were comparatively insoluble in the ether. The compound melted at 100° C. Analysis showed the absence of sulphur. The molecular weight using the freezing point method was found to be 162. The carbon and hydrogen analysis resulted in data indicating the molecular formula $C_{10}H_8O_2$. This compound, because of the structure of furfural polysulfide, must be difuryl ethylene.

Difuryl ethylene was tested as a rubber accelerator. No cure at any temperature was obtained. This indicates that the accelerating action of polythiofurfural is not due to the furane ring, but rather to the carbon and sulfur grouping.

Furfural was compared with pine tar and mineral oil as a rubber softener. It was found to produce about equal results, but is not exceptional, is difficult to use because of its volatility, and has the added disadvantage of being more expensive.

The polymerizing tendency of furfural and its compounds was one of the first characteristics of these materials to be observed. A striking illustration of this action was noted in an attempt to use furfural as an accelerator. The furfural was prepared by passing anhydrous ammonia into redistilled furfural. To this solution a quantity of hydrochloric acid was added, to determine whether the hydrochloride could be obtained directly. The mixture set to a gel in a few moments. An hour later it had become a firm hard piece of material having a black shiny appearance.

Various procedures were tested in an effort to get a stronger material. A furfural solution was prepared by passing anhydrous ammonia into technical furfural for an hour. The addition of relatively large amounts of acid was found to produce better material. Best results were obtained by using one third acid to two thirds furfural solution by volume. The use of redistilled furfural resulted in identical products. Assuming that all the ammonia was converted to furfural, about 19.3 per cent of the furfural combined with the ammonia. Because of the acid nature of the polymerizing agent, many objectionable features of the molding material were observed. To eliminate these difficulties, other polymerizing agents were tested.

In order to eliminate the introduction of water, hydrochloric acid gas was first tested, with poor results. Small amounts of various metallic

salts were added, but none of them resulted in the production of a solid material. Sulphur dichloride in amounts as small as five per cent was found to produce solid materials, but the mechanical strength was very low, and the product had a very objectionable odor. Sulfuric acid reacted very violently with the evolution of large amounts of gas and heat. Dilution of the acid before addition to the furfural solution was found to delay the reaction enough to prevent the formation of gas. The material was strong, had less tendency to crack, but was intensely acidic. Alkalies were found to have no polymerizing action, and exposure to silent electric discharge produced no change.

It was found possible to mix various fillers with the liquid material. Fillers tested were cornstalk and cob flour, wood flour, asbestos, and oat hull residue. Various articles were molded using mixtures of fillers and molding liquid.

Since acids were the only materials found which would produce hard materials, attention was turned to the problem of removing the acid after the material had solidified. Samples were placed in contact with flake caustic and ammonia, which resulted in a surface neutralization of the acid, but which did not affect the internal acid content. Samples were soaked in a variety of organic solvents without result other than a marked tendency of the sample to crack when removed from the solvent. The best method found for the elimination of acid was a slow drying or baking of the material.

The density of the material was found to be about 1.44. The color was always a jet black. The tensile strength of the material when mixed with a corn cob flour filler was around five hundred pounds per square inch. A high compressive strength was obtained, about thirty-five thousand pounds per square inch.

The volume resistivity of the material was found to be 2×10^{12} ohms per cc. When heated the material had the appearance of charcoal after being thoroughly calcined. It will not, however, burn with a flame. The ordinary acids and alkalies have no action on the material.

One of the articles molded in large quantities using the furfural molding liquid was golf tees. The molds used were drawn from glass tubing. At first the molds were filled by hand, but a small machine was developed which greatly facilitated this operation. After solidifying, the tees were hardened by baking. The estimated cost of production of these golf tees was \$1.05 per thousand. Other articles molded were buttons, tubes, and balls. The liquid was also tested as a binding material, and was tried out as a material for coal briquetting. A mixture of carborundum and the furfural molding compound was tested as a material for grind wheels, but was found to be too soft.

The molding material produced by the action of hydrochloric acid on a solution of furfural in furfural is not as yet a commercial product. It does, however, have definite commercial promises.

THE DISSOCIATION CONSTANTS AND ROTATIONS OF SOME ALPHA-SUBSTITUTED ETHYLAMINES¹

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Optical activity, the property of rotating plane polarized light, was first observed with quartz crystals in 1811. It was later observed that solutions of sugar and tartaric acid and turpentine vapor also possessed this property. Since that time a vast amount of work has been done on naturally occurring optically active substances and on synthetic compounds which may be resolved into active forms. The concepts of specific and molecular rotations were introduced about 1815 and have been used since that time to systematize rotation data. The effect of temperature and solvents on various optically active compounds has been investigated by many workers. It was noted about 1835 that the degree of rotation varied with the wave length of the light source. This discovery led to numerous investigations relating to dispersion data.

Very few successful attempts have been made to correlate the degree of rotation with the structure of the optically active substance, if we except the work of Hudson and others on sugar derivatives. Attempts made by Guye² and Crum Brown³ to calculate rotations from the mass of the radicals attached to the asymmetric carbon atom proved unsuccessful even for homologous series. Betti⁴ and Rule⁵, however, obtained very good correlations when they took into account the electrical properties of the radicals, as measured by dissociation constants and dipole moments. Betti compared rotations of about thirty aldehydo-aminic

derivatives of β -naphtholphenylamino methane, $\text{C}_6\text{H}_5\text{-C}(\text{H})(\text{C}_{10}\text{H}_6(\text{OH}))\text{-N}(\text{H})\text{=C-R}$, with

the dissociation constants of the acids, RCOOH , where R is a substituted phenyl radical. Rule compared rotations of a series of homogeneous 1-menthyl esters of monosubstituted acetic acids, $\text{XCH}_2\text{-COOH}$, with the dissociation constants of the acids. Betti and Rule pointed out that increasing the 'electronegativity' of one of the four radicals attached to the asymmetric carbon atom tended to increase the molecular rotation.

Werner Kuhn gave a mathematical treatment of optical activity in 1930⁶ which showed that vibrating electric oscillators, resonators, in molecules may bring about the rotation of plane polarized light. His

¹ Original thesis submitted June, 1935.

² Guye, *Compt. rend.*, **110**: 714 (1890).

³ Crum Brown, *Proc. Roy. Soc. Edinburgh*, **17**: 181 (1890).

⁴ Betti, *Gazz. chim. ital.*, **50** (II): 276 (1920).

⁵ Rule, *J. Chem. Soc.*, 1121 (1924).

⁶ W. Kuhn, *Trans. Farad. Soc.*, **26**: 293-308 (1930).

treatment is based on the conception that plane polarized light is composed of left- and right-hand circular polarized light. He concluded that "optical activity is a phenomenon of light refraction and, we shall find, in analogy with the law of additivity in molecular refraction, that the optical rotation of a compound is the sum of contributions of the various substituents, or, more accurately, of their absorption bands." It follows from Kuhn's work that the asymmetry of coupled absorption bands, or electrical oscillators, is a more fundamental property of optically active compounds than is the asymmetry of masses or structural groups.

In an attempt to clarify further the effect of varying radicals in optically active compounds, a series of α -substituted ethylamines were prepared and resolved. Secondary butylamine, α -benzylethylamine, α -p-tolyethylethylamine, α -phenylethylamine, and α -p-diphenylethylamine were prepared and resolved according to direction obtained from the literature and modifications of these methods. α -o-Chlorobenzylethylamine was prepared and resolved for the first time. Two separate preparations and repeated resolutions gave identical rotations. Micro Dumas nitrogen analyses were made on all new compounds and others were characterized by boiling points and derivatives. Rotations of the pure amines, of methyl alcohol, ethyl alcohol, and hexane solutions of the amines, and of the amine hydrochlorides in methyl alcohol were measured with a sodium arc as the light source.

If comparisons of rotations are to be made it is necessary to compare values for active forms having the same configuration. Leithe⁷ catalytically reduced α -phenylethylamine to α -cyclohexylethylamine and observed the dextro form of the former gave the levo form of the latter. He also observed that catalytic hydrogenation of d- α -benzylethylamine gave d- α -hexahydrobenzylethylamine. Reduction of d- α -o-chlorobenzylethylamine under pressure in the presence of palladium oxide catalyst was shown in this thesis to replace the chlorine with hydrogen without reducing the benzene ring. This configurational study showed that d- α -o-chlorobenzylethylamine gave d- α -benzylethylamine. Studies of this type involving only catalytic reductions are not likely to involve Walden inversions.

The molecular rotations of the amine hydrochlorides in methyl alcohol were observed to be lower than the molecular rotations of the amines. Some work was carried out to determine the effect of a change in pH on the rotation of the amines. A methanol solution of d- α -phenylethylamine was neutralized in steps with gaseous hydrogen chloride and the rotation and pH observed at intervals. The molecular rotation plotted against pH gave a curve similar to electrometric titration curves. The greatest slope in the rotation-pH curve, however, occurred at the pH for half-neutralization of the amine rather than at complete neutralization.

The dissociation constants of the α -substituted ethylamines mentioned above were measured in methyl alcohol in order to compare them with the molecular rotation of the amines. The method used was the determination of the hydrogen ion activity at half-neutralization of the amine with hydrogen chloride. Voltage values were measured by means of a hydrogen electrode and a calomel half-cell containing 0.1 formal sodium

⁷ Leithe, *Ber.*, 63: 800-865 (1930).

chloride in methanol. The calomel half-cell was standardized against silver-silver chloride electrodes at intervals of one or two weeks. Little variation in the e. m. f. of the calomel half-cell was observed over a period of six months. The method and technique used and the calculations involved in the determination of dissociation constants in methyl alcohol have been described by Goodhue and Hixon⁸.

When the radical, R, in the α -substituted ethylamines, $\begin{array}{c} \text{CH}_3 \\ | \\ \text{H}-\text{C}-\text{NH}_2 \\ | \\ \text{R} \end{array}$, was

varied, the molecular rotation of the pure active amines varied. Changing R from phenyl to cyclohexyl changed the molecular rotation from $+49.4^\circ$ at 15°C. to -4.06° at 15°C. that is, replacing the relatively 'electronegative' group, phenyl, by the relative 'electropositive' group, cyclohexyl, produced a marked change in optical activity even to the extent of changing the sign of rotation. Members of the series derived from radicals with intermediate electrical properties had molecular rotations between these two extreme values.

The dissociation constants of the α -substituted ethylamines, $\begin{array}{c} \text{CH}_3 \\ | \\ \text{R}-\text{C}-\text{NH}_2 \\ | \\ \text{H} \end{array}$,

in methyl alcohol, of the primary amines, RNH_2 , in water, and of the carboxylic acids, RCOOH , in water and the dipole moments of the chlorides, RCl , in benzene were compared with the molecular rotations of the α -substituted ethylamines. With but few exceptions a decrease in the dissociation constants of the amines, or an increase in the dissociation constants of the acids, or a decrease in the dipole moments of the chlorides correspond to an increase in the molecular rotations of the active α -substituted ethylamines.

It was concluded that there is a relationship between the electrical properties of the radical, R, as measured by dissociation constants and dipole moments, and the molecular rotations of the α -substituted ethyl-

amines, $\begin{array}{c} \text{CH}_3 \\ | \\ \text{R}-\text{C}-\text{NH}_2 \\ | \\ \text{H} \end{array}$. It was pointed out, however, that the relation is not a

simple one and no attempt was made to develop a quantitative relationship.

⁸ Goodhue and Hixon, *J. Am. Chem. Soc.*, 56:1329 (1934).

STUDIES ON THERMOPHILIC BACTERIA FROM COMMERCIAL CANE SYRUPS¹

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A study was made on 73 cultures chosen from 210 cultures of bacteria isolated (at 55° C.) from commercial cane syrups. On the basis of temperature relationships these could be subdivided into two groups, which included (a) 48 cultures which grew at 65° C. but not at 37° C., and (b) 54 cultures which grew at 37° C. and 55° C. but not at 22° C. and 65° C.

Determination of the morphological, cultural and physiological characteristics was made according to the methods suggested in the Manual of Pure Culture Study prepared by the Society of American Bacteriologists. All the cultures isolated and examined were rods, which were twice as long as wide. All cultures produced spores and were motile. In young cultures all the cells were Gram positive. Many of the cultures showed Gram negative cells after 12 to 24 hours incubation and in several, all cells were Gram negative after five days.

A brief characterization of the organisms was made using the method as outlined by the Descriptive Chart which accompanied the Society Manual of Pure Culture Study (1929). By use of the index number thus obtained, 15 groups are presented. Divisions within the groups are also pointed out.

None of the organisms produced gas in nutrient broth containing dextrose, lactose or sucrose. Acid production from 21 carbohydrates and alcohols in nutrient broth was determined. The ability to produce an acidity from the carbohydrate or alcohol in nutrient agar was determined, together with the ability to change the reaction produced from an acid to neutral or alkaline.

A study was made of 35 organisms as to their ability to utilize carbohydrates, alcohols and organic acids when grown on a synthetic medium containing the test material as the sole source of carbon.

The osmophilic relationships of each of the organisms was observed by growing it in a special medium with various concentrations of sucrose.

Work was presented to show the effect of the initial hydrogen ion concentration on the vigor of growth of the organisms. It was observed that the optimum hydrogen ion concentration for growth was generally between pH 6.3 and pH 7.0. One group, with an optimum between pH 6.3 and pH 7.8, showed the ability to grow well at pH 4.7. A new species described had an optimum of pH 5.0 and pH 6.0 and gave good initial growth at pH 4.7 and delayed growth at pH 7.8.

Comparative studies are presented on procedures recommended for the determination of nitrate and nitrite reducing ability of microorganisms. The method as outlined in the Manual of Methods is compared with that presented by ZoBell where the use of a semisolid medium containing nitrate is recommended.

Observations were made on the ability of selected organisms to

¹ Original thesis submitted December, 1934.

produce a positive nitrite test in nutrient media without a known source of nitrate. Evidence presented shows that the material causing the positive nitrite test after the culture had grown reacted like a nitrite. The conclusion that these organisms are able to produce a nitrite from ammonia or protein sources is withheld until such a time as a study can be made under the most rigid control.

Organisms studied included strains which are apparently identical with *Bacillus michaelisii* Prickett, *Bacillus thermoliquefaciens* Bergey, *Bacillus thermoalimentophilus* Weinzirl, *Bacillus terminalis* Migula var. *thermophilus* Prickett, *Bacillus thermoindiffrens* Weinzirl, *Bacillus aerothermophilus* Weinzirl, and *Bacillus thermoacidurans* Berry.

Another group of organisms showed a close relationship to *Bacillus fusiformis* Gottheil but because of the thermophilic characteristic the name *Bacillus fusiformis* Gottheil var. *thermophilus* var. nov. is proposed. A complete description of this organism is given.

The description of a group of organisms whose characteristics set them apart from all the other organisms reported in the literature is detailed. The organisms will grow well in media of relatively high hydrogen ion concentration (pH 4.7) and when grown in a synthetic medium with dextrose as the sole source of carbon, a distinct odor of acetic acid was produced. A distinctive acid odor was produced when the organism was grown in nutrient media containing carbohydrates. Characteristics observed in this group included reduction of nitrates, action on carbohydrates, action on litmus milk and production of acetyl-methyl-carbinol. The name *Bacillus oxyphilus* n. sp. is proposed for this organism. A complete description of this organism is given.

THE REVERSIBLE SPLITTING OF ORGANOMERCURIC CYANIDES WITH HYDROGEN CHLORIDE¹

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Gradations of electrical properties from one typical class of organic compounds to another suggest that some property other than type of structure may better serve as a basis for systematizing organic chemistry. A point of attack that naturally suggests itself is a study of the relatively permanent aggregates of atoms designated as radicals. If some fundamental property common to organic radicals exists, capable of serving as a classifying index, the assaying of this property should yield a more natural and adequate means of systematizing organic chemistry.

An examination of the ionization constants of organic acids, RCOOH , where R contains no ionizable group, shows that the constants range in value from about 10^{-6} to about 10^{-1} . The range of the amine constants is from about 10^{-14} to about 10^{-4} . The ionization constants of a series of amines can be arranged relatively to each other so as to give an orderly increase in their magnitudes. Corresponding to each constant there would be a radical. If to each radical there is assigned a number the condition that a functional relation exist between the ionization constants and the numbers designating the radicals is satisfied. Such a functional relation was established by Hixon and Johns². They demonstrated that organic radicals fall into a significant series on plotting as ordinates on a smooth arbitrarily drawn curve the logarithms of the ionization constants of a series of primary amines. At the point on the abscissa corresponding to $\log K$ for a given amine the radical was placed. This procedure fixed the radicals along the abscissa at points which must necessarily satisfy a functional relation. Upon plotting $\log K$ of the carboxylic acids against the radicals thus fixed along the abscissa a smooth curve was obtained. Further data on ionizations and equilibrium reactions in various solvents were found to lend themselves to this treatment.

Thus there is established for organic radicals a quantitative scale on which a radical has a characteristic constant value. This scale serves as the axis of an independent variable. In this systematization the guiding principle was that the only reliable information on affinity relations between chemical groups is given by the free energy change of reversible processes in which the influence of the groups is involved.

Attempts have also been made to systematize organic radicals in an electronegativity series on the basis of the irreversible splitting of unsymmetrical organomercuric and organolead compounds. The definition of the relative electronegativity of the radical R' in the compound RHgR' was given by Kharasch as follows³: "The group R' which presumably

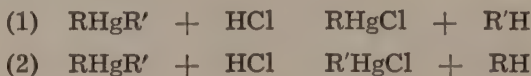
¹ Original thesis submitted December, 1934.

² Hixon and Johns, *J. Am. Chem. Soc.*, 49: 1786-95 (1927).

³ Kharasch and Flenner, *J. Am. Chem. Soc.*, 54: 674-92 (1932).

first dissociates from the mercury and then combines with the hydrogen ion in solution to form the hydrocarbon R'H is defined as the more electronegative of the two radicals; that is it has the greater attraction for electrons." The radicals studied in these cleavage experiments were arranged in a series. The interpretation placed on the series was that any radical is more electronegative than any below it and less electronegative than any above it. A comparison of this series with that obtained on the basis of ionization constants reveals that there is considerable difference in the relative positions of various radicals. The question naturally arises as to the comparative significance of the results of the two methods.

The experimental part reports the determination of the equilibrium constants of the reaction of some organomercuric cyanides with hydrogen chloride in ethyl alcohol, and the determination of the decomposition temperatures of some organomercuric cyanides. Equilibrium data on this reaction should serve as the basis of a critical comparison of the results that are to be expected from reversible and irreversible reactions of similar type. Whereas, in the splitting of RHgR' with HCl no reversal of the reaction is expected or observed due to the formation of hydrocarbons, in the reaction of RHgCN with HCl reversal is expected and observed due to the reactivity of HCN . Since no reversal occurs in the case of the RHgR' compounds the only practical means of evaluating the reaction is to determine the quantities of RHgCl and R'HgCl produced. The relative amounts of these compounds measure the relative rates of the two competitive reactions:



In the case of the reversible splitting of RHgCN with HCl the equilibrium constants serve as a measure of the free energy of the reaction.

The organomercuric cyanides used were prepared either by treating RHgBr or RHgI with silver cyanide, or by the reaction between $\text{R}_2\text{Hg} + \text{Hg}(\text{CN})_2$. The organomercuric cyanides were analyzed for nitrogen by a micro-Dumas analysis. The organomercuric chlorides were furnished by Dr. I. B. Johns. Absolute ethyl alcohol was used as the solvent for the reaction. The temperature of the reactions was controlled to $25^\circ \text{C.} \pm 0.1^\circ$.

Preliminary experiments on the reaction of RHgCN with HCl in alcohol indicated that the reaction proceeded to a great extent in the direction of the production of RHgCl and HCN as shown by the great decrease in the conductivity of a solution of HCl into which was introduced an equivalent quantity of RHgCN . Reversal of the reaction was evidenced by the development of a relatively high conductivity of a mixture of RHgCl and HCN . The equilibrium constants were determined in the following way. A solution of HCl of about 0.01 N. was prepared in alcohol and analyzed for chloride. The analysis consisted of adding excess standard silver nitrate, filtering off the precipitated silver chloride and titrating the excess silver by the method of Sharwood. A portion of this solution was diluted to 0.005 N. for running the reactions between RHgCN and HCl . Another portion was diluted over the range of concentration occurring in the reactions. The specific conductivities

corresponding to these dilutions were determined and a graph of concentration against conductivity constructed. To a 25 cc. volume of 0.005 N. HCl solution was added an equivalent amount of RHgCN and the resulting conductivity determined. A correction was made for the conductivity of the components RHgCl and HCN . The equilibrium concentration of HCl was then determined from the graph as the value corresponding to the corrected conductivity of the reaction. The equilibrium constant calculated from this value and the initial concentration of HCl. The reverse reaction between RHgCl and HCN was run similarly. On plotting $\text{Log } K$ for the various compounds RHgCN against radicals as fixed on the abscissa by the ionization of acids and amines a smooth curve was obtained.

The decomposition temperatures were determined by heating a small quantity of compound in a melting point tube and observing the temperature at which blackening occurred. The graph of the decomposition temperatures against the radicals was a curve passing through a minimum near the benzyl radical.

These results show that the reversible splitting of RHgCN with HCl measures a different property of the R-Hg bond than does the irreversible splitting of RHgR' with HCl. A correspondence was shown to exist between the results of the thermal decomposition of RHgCN compounds and the irreversible splitting of unsymmetrical organomercurials with HCl. Both the decomposition of unsymmetrical organomercurials with HCl and the thermal decomposition of organomercuric cyanides apparently measure the stability of the C-Hg bond involved in the decomposition.

It was pointed out that on the basis of the use of refraction as a measure of the deformability of electron shells and as a measure of the electron constraint the constraining force of a radical on the group to which it is attached may be measured by means of refraction.

SOME PHYSICO-CHEMICAL STUDIES OF ORGANOMETALLIC AND FURAN COMPOUNDS¹

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A. RELATIVE REACTIVITIES OF HALIDES AND SUPER-AROMATIC PROPERTIES OF FURAN

The relative reactivities of some furan halides were determined by a study of reaction velocities with potassium iodide in an acetone solution. The procedure used was essentially that of Conant and Kirner².

The values observed for R (R = relative reactivity at 50°, *n*-butyl chloride = 1) were: 2-furylmethyl chloride 3,184, 5-nitro-2-furylmethyl chloride 12,708, 2-furoylmethyl chloride 98,230, 2-tetrahydrofurylmethyl chloride 0.025, γ -(2-furyl)-propyl chloride 2.51 and γ -(2-tetrahydrofuryl)-propyl chloride 1.33. Qualitative observations showed 2-furoyl chloride to be less reactive than benzoyl chloride. Halogens which are directly attached to the furan ring are extremely inert.

The negativity (or aromaticity) of the radical appears to be the most important factor in the reaction of the halide with potassium iodide. The reactivity of the halogen in the series ACH_2X will increase with an increase in the negativity of the aromatic nucleus; however, in the series AX the reactivity of the halogen decreases with an increase in the negativity of the nucleus A. When the aromaticity of the furan nucleus is essentially destroyed by complete nuclear hydrogenation there is a marked drop in reactivity of lateral chlorine.

B. PARACHORS OF SOME FURANS

The purpose of this investigation was to gain some more definite information concerning the structure and the dynamic behavior of the furan nucleus.

The parachors of furan and a number of its derivatives were determined. The method was essentially that of Sugden³. The observed values of these parachors were: furan 160.4, 2-methylfuran 199.8, 2,5-dimethylfuran 240.6, 2-nitrofuran 220.8, 2-bromofuran 212.6, 2-furfuryl alcohol ("water soluble form") 216.9, 2-furfuryl alcohol ("water insoluble form") 216.2, 2-furfuryl methyl ether 260.8, 2-tetrahydrofurfuryl ethyl ether 321.3, 2-furfural 212.5, ethyl 2-furoate 309.6 and 2-furfuryl 2'-furoate 398.9.

These results are in support of the classical diolefine formula or structures related to it. The ethylene oxide, formula is probably not pres-

¹ Original thesis submitted December, 1934.

² (a) Conant and Kirner, *J. Am. Chem. Soc.*, 46:232-252 (1924);

(b) Conant and Hussey, *ibid.*, 47:476-488 (1925);

(c) Conant, Kirner and Hussey, *ibid.*, 47:488-501 (1925).

³ Sugden. "The Parocher and Valency," London, 1930.

ent to a significant extent. It is, of course, impossible within the limits of accuracy for measuring parachors to definitely and rigorously exclude any formula. The best that can be hoped for is an idea of predominant forms. It still appears altogether probable that furan consists of a dynamic equilibrium of a number of structures, some of which are more useful than others in interpreting specific reactions and properties.

C. IONIZATION CONSTANTS OF SOME ACIDS OF THE FURAN SERIES AND SUPER-AROMATIC PROPERTIES OF FURAN

The negative character of the furan nucleus has been described in terms of the relative ease of splitting of mixed organolead compounds; ease of substitution in the furan nucleus; and relative reactivity of the halides. A further manifestation of the activating influence (aromaticity) of the furan nucleus is to be found in the strength of the carboxylic acids as compared to the corresponding acids of the benzene series.

The ionization constant was calculated from the pH of the half neutral solution of the acid. The quinhydrone electrode and the saturated calomel electrode were used in determining the pH. The acids studied and their ionization constants ($\times 10^5$) were: 3-chloro-2-furoic 204.1, 5-chloro-2-furoic 147.4, 5-bromo-2-furoic 144.3, 5-iodo-2-furoic 116.0, 3,4-dichloro-2-furoic 400.3, 3,5-dichloro-2-furoic 377.4, 4,5-dichloro-2-furoic 248.6, 3,5-dibromo-2-furoic 326.8, 5-nitro-2-furoic 870, 5-methyl-2-furoic 38.12, 2-methyl-3-furoic 2.94, 2,4-dimethyl-3-furoic 2.79, 2,5-dimethyl-3-furoic 2.296, mucobromic 5.26, furylacrylic 3.83, 2-furoic 75.2, 3-furoic 11.3, and thiophene-2-carboxylic 34.26.

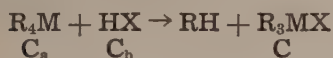
The strength of the substituted furoic acids was correlated with the nature and position of the substituents. The ionization constants of the acids were also correlated with the rates of alkaline hydrolysis of the esters studies by C. W. Bradley and with the comparative reactivity of the halides containing the same number of carbon atoms.

D. RELATIVE REACTIVITY OF SOME ORGANOMETALLIC COMPOUNDS

A quantitative method has been developed for the study of the comparative reactivity of some organometallic compounds of some of the elements in the second, third, fourth and fifth groups. The method consists of splitting the organometallic compound (in solution) with an acid of suitable strength and solubility. An excess of the organometallic compound is used and the course of the reaction is followed by extracting the unreacted acid with water and titrating it.

The relative reactivities of the ethyl and phenyl derivatives of lead, mercury, and tin, and the phenyl derivative of bismuth have been determined by a study of the reaction velocities with trichloroacetic acid and hydrogen chloride in chloroform.

In calculating the results a modification of the second order reaction velocity expression was used:



$$k = \frac{1}{t(r-1)b} \log \frac{r-z}{r(1-z)}$$

The factor 2.303 was omitted in all cases.

t = time in hours;

$$r = \frac{C_a}{C_b};$$

b = concentration of HX in moles per liter;

z = fraction of HX reacted in time t .

The following data summarize the comparative reactivity of these compounds: tetraethyl lead 6, tetraphenyl lead 56, diphenylmercury 57, triphenylbismuth 40, ethyltriphenyl-lead 2,000. These data represent the velocity of splitting with trichloroacetic acid at 25° C. With hydrogen chloride the constants were: tetraethyl-lead 410 (10°), tetraethyltin 6.9 (25°), tetraphenyltin 75 (10°) and diethylmercury 30 (10°).

In every case the phenyl derivative is more reactive than the corresponding ethyl derivative and the splitting with hydrogen chloride is more rapid than with a weaker acid.

The effect of numerous catalysts was studied. Of these, diatomaceous earth and oxygen (or oxidation products) were found to greatly increase the rates of reaction.

SOME BACTERIOLOGICAL AND CHEMICAL EFFECTS OF CALCIUM AND MAGNESIUM LIMESTONES ON CERTAIN ACID IOWA SOILS¹

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The comparative value of calcium and magnesium limestones has been a problem of considerable interest to agronomists for many years, and it has even caused some controversy among limestone dealers as evidenced by their claims of superiority for their particular products. This interest and controversy have resulted from the early experimental work conducted mainly by Loew on the functions of lime and magnesia in plant nutrition and growth. From this work an hypothesis was formulated which stated that calcium and magnesium are toxic to plants when either occurs in excess of a definite ratio of lime to magnesia.

In their work, Loew² and May³ in 1901 experimented with water, sand, and soil cultures treated with the sulfate, the nitrate and the carbonate of calcium and magnesium. The plants grown in the cultures were barley, oats, wheat, conifers, privet, beans, and tobacco. The results obtained from these investigations showed that magnesium was toxic to the plants when it occurred in great excess over the lime content. It was also noted that the noxious effects were counteracted by an excess application of calcium.

With these results before them many investigators took up the task of determining whether or not Loew's hypothesis is tenable. As a result considerable experimental work has been done along this line during the past thirty-four years. In general, the many investigators have given results which may be classified into three groups, (1) those supporting Loew's hypothesis, (2) those showing little or no support for its tenability, and (3) those which are related to the subject of the functions of lime and magnesia in plant growth, but give little or no evidence either in support, or contradiction, of the hypothesis in question.

Because of these differing viewpoints and since many soils contain large quantities of both calcium and magnesium carbonate it is desirable that no prejudice be developed as to the comparative agricultural value of these two materials. Hence, the object of this investigation was to determine and to compare the effects on soil conditions and plant growth of calcium and magnesium limestones applied to certain Iowa soils in different amounts and various degrees of fineness.

In order to determine the effects of a high-calcium and a high-magnesium limestone on the soil conditions and plant growth the experimental work was divided into two series, one series consisting of greenhouse experiments using Carrington loam and Tama silt loam and the

¹ Original thesis submitted June, 1935.

² Loew. U. S. D. A. Bur. Plant Indus. Bul. 1. 1901.

³ May. U. S. D. A. Bur. Plant Indus. Bul. 1. 1901.

other series consisting of laboratory experiments employing Grundy silt loam.

SERIES I. GREENHOUSE EXPERIMENTS

In series I the soils were placed in glazed stone pots and divided into two series, one series being treated with calcium limestone and the other with magnesium limestone. The limestones were applied in amounts as follows: (1) no lime, (2) $1\frac{1}{2}$ tons per acre, (3) 3 tons per acre, (4) 6 tons per acre. Two pots of each treatment were cropped to wheat and red clover and the other two were fallowed and used for laboratory analyses. The high-calcium limestone used in this experiment contained 93.50 per cent calcium carbonate, 1.65 per cent magnesium carbonate and was 93.54 per cent pure. The high-magnesium limestone contained 49.20 per cent calcium carbonate, 21.20 per cent magnesium carbonate and was 90.56 per cent pure. In adding the limestones to the soils, different fractions of the magnesium limestone were mixed together in order to obtain a composite material of the same mechanical composition as the calcium limestone employed. This was considered desirable in order that the two limestones, differing in chemical composition, might be studied under comparable conditions in so far as fineness of division is concerned.

The soils of the fallow series were sampled at the end of $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 3, 4, and 5 months after the experiment was started. The following analyses were made: (1) Moisture content (2) pH (3) lime requirement (two methods) (4) exchangeable hydrogen (5) exchangeable calcium (6) exchangeable magnesium (7) total exchangeable bases (8) exchange capacity (9) degree of saturation (10) nitrate content and nitrifying power. Crop yields were determined on the soils growing wheat and red clover.

In general, the results obtained from the experiments in this series show that calcium limestone was slightly more effective than the magnesium limestone during the early periods of the experiments, and that the magnesium limestone was equally as effective as the calcium limestone at the end of the experiment. On the basis of the data obtained it is believed that in actual practice where the limestones are allowed to react with the soil acids over a relatively long period of time the calcium and magnesium limestones will give rather similar results.

It has been found by various investigators that if the supply of magnesium in the soil is extremely large toxic effects may result from the additions of magnesium limestone. Inasmuch as no large difference was obtained in the effects of the two limestones in these experiments it is apparent that the supply of magnesium in the soil is sufficient to meet the needs for plant growth. It is also apparent that the soils studied do not contain too large a supply of magnesium as additional applications of a large amount of magnesium limestone did not produce deleterious effects.

SERIES II. LABORATORY EXPERIMENTS

In this series of experiments 100gms. of Grundy silt loam were placed in each of 376 tumblers and mixed with calcium and magnesium limestone. The limestone treatments were made in triplicate, using 10-, 20-, 40-, and

100-mesh limestone at rates of $1\frac{1}{2}$, 3, and 6 tons per acre. Pure calcium and magnesium carbonate were also employed, being applied at the same rates as the limestone applications. The calcium and magnesium limestones were of the same source as those used in the experiments of series I.

In addition to the limestone treatments two tumblers of soil were treated with ammonium sulfate for nitrification of the ammonium ion. The nitrifying power and the hydrogen-ion concentration was determined at weekly intervals. The soil not treated with ammonium sulfate was analyzed for hydrogen-ion concentration and nitrate content.

The data from this series of experiments indicate that the coarser calcium and magnesium limestones gave similar results, while the finer grades of calcium limestone were somewhat more effective than those of the magnesium limestone during the early periods of the experiment. At the end of the experiment, however, there was very little difference between the two limestones. This conclusion is in accord with that drawn from the experiments in series I.

It was further noted that the finer limestones had a much larger effect upon the hydrogen-ion concentration and the nitrifying power of the soil than did the coarser limestones.

The pure carbonates of magnesium and calcium were somewhat more effective in neutralizing the acidity of the soil than were the limestones. In general, very little difference was found between the effects of the pure carbonates and the 100-mesh limestone on the nitrifying power of the soil.

THE EFFECTS OF LIMING ON THE LIBERATION OF POTASSIUM IN SOME IOWA SOILS¹

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Considerable attention has been paid in the past to the effects of calcium compounds in liberating available potassium in soils. A review of the more recent literature, however, reveals a contradictory idea of the influence of calcium additions upon plant food potassium. The early investigators and most of the textbooks on soils adhere to the viewpoint that the addition of lime to soils results in the liberation of available potassium through chemical reaction. The earlier investigators showed that additions of calcium to the soil affected the solubility of potash and even made potash fertilization unnecessary. It was pointed out as early as 1847 that lime additions furnished an invaluable means of liberating from the soil the alkalis which are indispensable to the existence of plants.

Many of the more recent investigators, on the other hand, have reported entirely different results upon the study of the lime-potassium relationship in soils. Data have been presented which show that lime did not liberate potassium but in some cases it even depressed the availability of potassium. Experiments with various crops, such as wheat and barley, revealed the fact that liming may materially reduce the solubility of both native and added potassium. The lime may be so active in depressing the solubility that in time the soil may become deficient in available potassium, especially for certain crops.

The exact reason for this depressive effect is not known, although many suggestions have been made. It has been pointed out that additions of lime to a soil decrease considerably the hydrolytic process that normally occurs. It has been found that the greater the concentration of carbonates and bicarbonates, the less will be the hydrolytic breakdown of the potash-bearing colloidal complex. If this is true, then soils having high concentrations of certain calcium compounds would contain only small quantities of available potassium. Another explanation given by some investigators is that as soon as the potassium is liberated it is absorbed immediately by the lower layers of soil and may be out of the feeding zone of some plants. It has been pointed out further that although it may be demonstrated that lime will liberate potassium from pure potassium minerals, it is quite likely that the same process will not take place in soils. It has also been suggested that since the soil contains clay, any potassium liberated by calcium may be reabsorbed by the finely divided material and kept from going into solution. Some investigators have concluded, however, that the potassium liberated is not merely absorbed by the clay fraction but reverts to a difficultly available form of potassium, usually resembling muscovite.

¹ Original thesis submitted March, 1935.

It appears that one of the above mentioned conditions may exist in the high-lime soils of Iowa. The high-lime soils, or so-called "alkali" spots of Iowa, have been found to contain high concentrations of both calcium carbonate and calcium bicarbonate, and these compounds are definitely associated with the unproductiveness of the soils. Various investigators have reported beneficial effects from the use of potassium fertilizers on such soils and have recommended their use. The purpose of this investigation was to study the fixation of potassium in soils and to determine the effect of lime on the liberation of potassium in some Iowa soils.

The investigational work reported here consisted of a number of laboratory and greenhouse experiments. Six studies were made, namely, (a) the analysis of 12 high-lime soils, (b) the nitrogen, the phosphorus, and the potassium content of corn plants grown on normal and on high-lime soils, (c) the effect of calcium carbonate on some exchange reactions in acid, neutral and basic soils, (d) the effect of certain calcium compounds on the exchangeable and available potassium in acid and basic soils, (e) the absorption of potassium and calcium by inorganic colloids, (f) the biological fixation of potassium in several Iowa soils.

All of the high-lime soils studied contained relatively large amounts of carbonates, nitrogen and potassium. However, tests for available potassium showed that 11 of the 12 soils studied were either in need or would respond to potash fertilization. In general, there seemed to be a direct relationship between high carbonate content of the soil and low amount of available potassium. Soils containing 20 per cent or more of carbonate were deficient in available potassium regardless of the amount of total potassium they contained.

The exchange capacity of the high-lime soils was larger than that of the acid Tama silt loam and the exchange complex of the high-lime soils contained a larger proportion of organic colloids than that of the Tama silt loam. The exchange complex of the high-lime soils was saturated with bases, principally calcium, whereas, the exchange complex of the Tama silt loam was unsaturated with respect to bases and contained larger amounts of exchangeable potassium. The addition of 6 tons per acre of calcium carbonate to the acid Tama silt loam in greenhouse experiments resulted in the saturation of the complex, and the depression in the availability of potassium. In other words, the addition of lime to an acid Tama silt loam brought about a condition in this soil after a period of 6 months which was similar with respect to exchangeable potassium to that existing in the high-lime soils. However, the addition of calcium carbonate, calcium chloride or calcium hydroxide to Tama silt loam in laboratory experiments liberated potassium from the exchange complex and brought about a significant increase in available potassium when the tests were made 21 days after treatment. The soils and the measurements made were the same in both sets of experiments and the conditions of the experiment were the same, except that in the greenhouse experiments, the soil was treated 6 months before the exchangeable and available potassium were determined and in the laboratory experiments the determinations were made 21 days after the treatment of the soils. Apparently some reaction took place during the period between treatment of the soils in the greenhouse and the time the tests

were made to change the effect of the added calcium compounds. This apparent change in the effect of calcium salts on potassium availability was demonstrated to be partly biological in nature. The data showed that sterile, inoculated soils usually contained less available potassium after a given incubation period than sterile uninoculated soils and that the addition of calcium carbonate increased this depression in available potassium. Whether the microorganisms assimilated the potassium or exerted a protective effect on the complex, preventing the exchange of the potassium by the calcium is not known. However, the conditions of reaction and organic matter supply in the high-lime soils are optimum for maximum microbiological activity under favorable conditions of moisture and temperature.

The data obtained indicate that the depressive effect of calcium on the availability of potassium in soils may be due in part to the action of microorganisms. Although no experimental evidence was obtained, some of the repressive effect of calcium on the availability of potassium in high-lime soils is, no doubt, also due to the depressed hydrolysis of the primary potassium bearing minerals and according to the law of mass action and chemical equilibrium some of the available potassium may revert to an insoluble form.

STUDIES ON THE GENUS AEROBACTER¹

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A study was made on 251 cultures of the genus *Aerobacter*. In various phases of the investigation 7 to 52 strains of the genus *Escherichia* and 4 to 70 strains of the genus *Citrobacter* were employed for purposes of control and comparison.

To ascertain the incidence of *Aerobacter* strains in human feces, 162 samples (75 male and 87 female) were examined. Of these 46 or 28.4 per cent yielded the cultures sought.

Studies of the characteristics of bacteria of the genus *Aerobacter* as compared to the characteristics of the genera *Escherichia* and *Citrobacter* indicate:

1. On eosine methylene blue agar (Levine formula) colony characteristics of *Aerobacter* strains markedly differed from characteristics of colonies formed by *Escherichia* and *Citrobacter* strains. Of the 251 *Aerobacter* strains studied 237 or 94.4 per cent would probably have been detected as such by one with some experience. The *Citrobacter* strains formed colonies which more closely resembled the typical *Escherichia coli* than the typical *Aerobacter aerogenes*.

2. With regard to the differentiation of the genus *Aerobacter* from other genera of the colon group by physiological characteristics it was found that the Voges-Proskauer reaction was perfectly correlated with the methyl red test and better correlated with the fermentation of sodium malonate than with growth in citric and uric acid media, production of H_2S from Difco proteose peptone, and fermentation of cellobiose and alpha-methyl-d-glucoside.

3. Observations of character of growth of colon bacteria in media containing various iron salts showed that the function of the salts in question was influenced by the constituents of the medium. Ferric citrate served as a reactant with aesculetin to form ferric aesculetin in one medium and as an indicator for the detection of H_2S production in another medium. Ferric ammonium citrate was shown to be the source of ferric ion to form $Fe(OH)_3$.

4. The use of high temperatures for differentiation of the genera of the colon group was shown to give some correlation between the type of organism and gas production when carried out in the ordinary manner, that is, inoculated at low temperature and placed in an incubator of 46° C. With the temperature of the medium raised before inoculation and maintained at 45.5-46° C. the correlation between the type of organisms and gas production was not sufficiently great to warrant incubation temperature as a diagnostic or differential test.

¹ Original thesis submitted December, 1934.

5. The reduction of methylene blue by lactose broth cultures was found to be an unreliable criterion for differentiating the genera of the colon group.

6. Potentiometric measurements of oxidation-reduction potentials developed by lactose broth cultures of colon bacteria did not offer a perfect means of differentiating the genera of the colon group.

7. The rate of growth and activity of cells were shown to be important factors in determining the potentials developed by *Aerobacter-Escherichia* lactose broth cultures exposed to air.

8. A rapid and reliable routine test for detecting the production of acetyl methyl carbinol in six hour buffered glucose peptone water cultures is described. The technic involves the use of a creatine-KOH (0.3 per cent ceatine dissolved in 40 per cent KOH) solution.

Investigations on characteristics of the 251 *Aerobacter* culture indicated that 88 closely resembled *Aerobacter aerogenes*, 98 closely resembled *Aerobacter oxytocolum* and 65 closely resembled *Aerobacter cloacae*.

Based upon a statistical study, the classification of 251 sucrose positive *Aerobacter* strains shows the presence of four species. These are *Aerobacter aerogenes*, *Aerobacter oxytocolum*, *Aerobacter cloacae* and *Aerobacter Amesii*.

A description of the new species (*Aerobacter Amesii*) is given.

THE PREPARATION OF ERYTHROSE AND SOME OF ITS DERIVATIVES¹

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Little attention has been paid to the four carbon sugars because of their inaccessibility. All methods of preparation result in low yields of impure products. Since the tetroses have been so much neglected little is known of their physiological significance. The tetroses are also of interest on account of their chemical properties. The four carbon sugars can possess no larger than a five membered ring and derivatives with such furanose rings are known to be very reactive.

Erythrose has been prepared by Wohl's², Ruff's³ Weermann's⁴, and Zemplen's⁵ methods of degradation. Since the results secured by the applications of these procedures were unsatisfactory a new degradation method was investigated. The method selected had been utilized previously by Freudenberg⁶ in the preparation of d-threose.

l-Arabinose was used as the starting material for the new degradation. The method involved the following steps: arabinose \rightarrow acetobromo arabinose \rightarrow diacetyl arabinal \rightarrow arabinal \rightarrow erythrose. The real degradation reaction came in the splitting of the unsaturated arabinal by means of ozone. The ozone splitting of unsaturated sugars had been used only as a means of proving the positions of the double bonds until Freudenberg's⁶ experiments on the preparation of d-threose.

The formation of erythrose by the cleavage of the ethylenic linkage in arabinal was proven by the preparation of erythrosazone. However, the compound formed in the greatest amounts was 4-formyl erythrose. The treatment of the ozonization product with methyl alcohol containing hydrochloric acid produced the dimethyl acetal of 4-formyl erythrose. The dimethyl acetal was never secured in a pure condition, however, the presence of this compound in the impure product was shown by the methoxy content, rotation, changes in rotation on acid hydrolysis, instability, qualitative detection of formic acid, and the removal of the formyl group by alkali. A third product from the action of ozone on arabinal was separated in the form of a crystalline methyl glycoside. The analysis of this glycoside identified it as a methyl desoxypentoside.

The reaction of 4-formyl erythrose in an aldehyde rather than ring structure showed that three or four membered rings were not voluntarily formed. The reactions of 3,4,5,6-tetrabenzoyl glucose have shown

¹ Original thesis submitted March, 1935.

² Wohl, A., Ber., 32:3666 (1899).

³ Ruff, O., and Meusser, A., Ber., 34:1366 (1901).

⁴ Weermann, R. A., Rec. trav. chim., 37:15-61 (1917).

⁵ Deulofeu, V., and Selva, R. J., J. Chem. Soc., 1929, 225.

⁶ Freudenberg, W., Ber., 65:168 (1932).

likewise that three membered rings were not voluntarily formed⁷. Sugar derivatives with five and six membered rings have been known for a long time and recently compounds with seven membered rings (septanoses)⁸ were announced. The action of 4-formyl erythrose was in agreement with the prediction based on the tension theory that the five, six, and seven membered rings would be stable and the three and four membered rings unstable.

Acetone methyl erythroside was prepared by treating the product from the ozonization of arabinal with acetone and methyl alcohol containing sulfuric acid. The sulfuric acid was neutralized with an excess of calcium hydroxide which not only removed the acid but also the formyl group from the erythrose. By repeating the treatment several times good yields were secured. Acetone methyl erythroside was stable and showed no tendency to lose methyl alcohol and form thereby a reducing compound. The stable methoxy group in acetone methyl erythroside was very much unlike the labile methoxy groups of 4-formyl erythrose dimethyl acetal.

Acetone methyl erythroside was hydrolyzed by dilute sulfuric acid to give unsubstituted erythrose. This reaction was carried out under mild conditions so that only a very small amount of erythrose was decomposed. The sugar sirup obtained had a very slight yellow color. The rotation of the erythrose had an initial value of $[\alpha]_D = +11.5$ and an equilibrium value of $[\alpha]_D = +30.5$. These specific rotations are approximately the same as have been reported previously for 1-erythrose prepared by other methods.

Several new sugar derivatives which occurred as by-products in preliminary reactions were also investigated. These products included a new acetobromo arabinose, a tetraacetyl desoxypentose disaccharide and its deacetylated parent sugar, and dihydro arabinal.

The new acetobromo arabinose was obtained from the mother liquor remaining after the separation of the common β -acetobromo arabinose. The suspicion that the new compound was α -acetobromo arabinose was disproved by its analysis. The bromine analysis agreed with the values calculated for a pentaacetylbromo instead of a triacetyl bromo arabinose like the common β form. The carbon and hydrogen analysis also indicated that the compound was a pentaacetylbromo derivative. The acetyl determinations showed that the new compound required more sodium hydroxide to neutralize the acids formed than would have been needed, if the sample had been made up simply of acetic and hydrobromic acids. The large amount of alkali neutralized could only be accounted for by the formation of acids from the arabinose part of the molecule. This result indicated that the arabinose was present in a reactive form, therefore an open chain structure was suggested for the compound.

The tetraacetyl desoxypentose disaccharide was formed as a by-product in the reduction of β -acetobromo arabinose. The disaccharide possessed two crystalline forms one melting at 167-169° and the other at 185.5°. Neither of the two forms contained any solvent of crystallization and both possessed the same rotation in chloroform solution. The

⁷ Brighl, P., Mühlischlegel, H., and Schinle, R., Ber., 64:2921 (1931).

⁸ Micheel, F., and Suckfüll, F., Ann., 502:85-98 (1933).

compound would not reduce Fehling's solution until after acid hydrolysis, thus showing that the two monosaccharide units were linked through their reducing groups. The nature of the compound was established by molecular weight determinations, carbon and hydrogen analysis, and acetyl determinations. The acetyl determinations showed the presence of four acetyl groups which would allow two acetyls for each monosaccharide group. The desoxy groups were thought to be most likely situated on the second carbon atoms, therefore the compound probably was tetraacetyl 1-ribodesosido 1-ribodesoside. The acetyl groups were removed from the tetraacetyl disaccharide by means of barium hydroxide and the unsubstituted sugar was isolated as a crystalline compound. The melting point was 177-180°, the compound undergoing some decomposition during the heating. The carbon and hydrogen analysis agreed with the values calculated for a desoxypentose disaccharide. The acetyl free compound also did not reduce Fehling's solution until after acid hydrolysis.

Dihydro arabinal was formed by the reduction of arabinal in alcohol solution using palladium black as a catalyst. The reduced compound was a colorless sirup that distilled at 83-85° under a pressure of 1 mm. The dihydro arabinol was a stable compound and possessed a rotation in water solution of $[\alpha]_D = +48.3$.

THE STORAGE AND DISTRIBUTION OF COPPER AND IRON IN THE RAT¹

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Analyses of the copper and iron content of the tissues of many species of animals under varying conditions are to be found in the literature, but these analyses are difficult to compare because of the lack of similarity of plan by the several investigators. Not only the type of experimental animal used and the age and condition of the animal, but also the type of diet and the method of analysis employed, varied markedly. The purpose of this thesis was to provide a fundamental and systematic study, under controlled conditions, of the storage and distribution of copper and iron in the whole rat and in its various tissues, as influenced by age, sex, length of experiment, amount of copper and iron in the diet, and some abnormal conditions such as anemia and starvation.

The animals were fed a whole milk ration with varying amounts of copper and iron added in the form of copper sulfate and ferric chloride, respectively. When very large amounts (5 to 30 mg.) of copper were fed, the necessary amount of copper sulfate solution was thoroughly mixed with a weighed amount of the Steenbock stock ration and fed to the animals in place of the milk diet. All analyses for copper were made by the sodium diethyldithiocarbamate method and the potassium thiocyanate method was used for the iron determinations. In the case of the tissues, the copper and iron determinations were made on the same sample.

The per cent of copper and iron in rats receiving milk together with 0.05 mg. of copper as copper sulfate and 0.5 mg. of iron as ferric chloride daily was found to decrease with age, although the total number of milligrams of copper and iron present actually increased. Excess amounts of copper were added to the milk and iron ration in dosages varying from 0.1 to 2.0 mg. daily. When 0.1 to 1.0 mg. of copper were administered, a point of maximum storage of copper occurred after a time interval which was shortened by the larger dosages. When 2.0 mg. of copper were fed, both copper and iron showed a maximum storage during the first month, minimum retention in the second month, and subsequently increasing values. Five milligrams of copper added daily to the Steenbock stock diet produced in the animals the same type of storage as 2.0 mg. of copper administered with a milk and iron diet. When 15 and 30 mg. of copper were fed in the Steenbock ration, the copper content of the animals continually increased and the rats finally died. The female rats exhibited a distinct tendency to store a larger percentage of copper and iron than did the males.

The amount of copper in the diet did not permanently affect the retention of iron in healthy rats. However, when the animals were fed milk plus 0.5 mg. of copper with varying amounts of iron daily, it was found

¹ Original thesis submitted June, 1935.

that increased iron tended to increase the copper storage and decrease that of iron. When rats were fed milk with 0.5 mg. of copper and 5.0 mg. of iron daily over a period of time, the copper storage reached a maximum at three months and the iron storage decreased, except in the oldest animals.

Anemic animals were found to store copper and iron very rapidly. The retention of copper was increased when the copper dosage was made larger, if iron was administered at the same time. The retention of iron also increased with the larger copper dosages. Starvation did not greatly deplete the copper and iron reserves.

Seventeen organs and tissues were studied in growing rats, adult rats, and those approaching middle age, on two different levels of copper. These tissues included the blood, long bone of the leg, incisor teeth, striated, smooth and cardiac muscles, brain, kidney, skin and hair, lung, liver, spleen, suprarenal and thyroid glands, pancreas, ovary and testis. The copper and iron contents of blood, the long bones, striated muscle, and skin and hair remained constant under the experimental conditions. The copper content of the brain, lung, spleen and testis, and the iron content of the incisors, smooth muscle, kidney and liver were likewise constant.

Time decreased the iron content of cardiac muscle, suprarenal and thyroid glands, and the ovary. The iron content was increased by copper administration in the case of cardiac muscle, brain and thyroid gland; and it was decreased in the lung, spleen and ovary. Temporary increases in the per cent of iron of the pancreas and testis were caused by copper feeding.

The copper content of the incisors, smooth and cardiac muscles, suprarenal and thyroid glands, and pancreas was diminished by time. Large increases of copper took place in the ovary as the animal matured. Increased copper administration had the effect of increasing the copper content of the kidney, liver, suprarenal gland, and ovary; and it had the effect of diminishing the percentage of copper in smooth and cardiac muscles and the thyroid gland.

THE EFFECTS OF CALCIUM CHLORIDE UPON THE HYDROLYSIS OF PURE COMPOUNDS OF CEMENT¹

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When Portland cement is mixed with water it hardens or "sets" and becomes artificial stone. Various substances have been recommended and used for regulating this setting process but today the only materials used as admixtures are CaSO_4 and CaCl_2 . The presence of CaSO_4 not only retards the setting process but also gives an increase in strength of the resulting cement. Calcium chloride is unlike CaSO_4 in its action. By using three parts or less of CaCl_2 to 100 parts of Portland cement a high early strength and a speedier curing cement is obtained. The exact role of CaCl_2 in speeding up this setting process has been a subject for a great deal of research. Several theories have been presented regarding its action but as yet no very satisfactory answer has been given.

The purpose of this research was to study the effects of CaCl_2 upon the pure bodies that go to make up a normal cement clinker. If the effects of CaCl_2 upon the pure compounds are known, its action upon cement itself can be much better understood.

The study of a normal Portland cement clinker by the phase equilibria and X-ray methods shows that the principal constituents are $3\text{CaO} \cdot \text{SiO}_2$ and $\beta 2\text{CaO} \cdot \text{SiO}_2$. There is also normally present in smaller amounts $3\text{CaO} \cdot \text{Al}_2\text{O}_3$, $4\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot \text{Fe}_2\text{O}_3$, MgO and a trace of free lime.

EXPERIMENTAL

The pure compounds studied in this research were $3\text{CaO} \cdot \text{SiO}_2$, $3\text{CaO} \cdot \text{Al}_2\text{O}_3$, $\beta 2\text{CaO} \cdot \text{SiO}_2$ and $\gamma 2\text{CaO} \cdot \text{SiO}_2$. The materials used in preparing these compounds were CaCO_3 , Al_2O_3 and SiO_2 . These materials were ground to pass a 200 mesh sieve and analyzed before being used.

The pure constituents were prepared by mixing weighed quantities of the raw materials in the proper proportions and burning in a gas-fired crucible furnace. The high temperatures required were obtained by means of a special blast burner and a gas enriched with benzene. The resulting compounds in each case were ground to pass a 200 mesh sieve, mixed and reheated to insure homogeneity. The product obtained after the second burning was ground and mixed but analyzed before reheating. In case the analysis did not check the theoretical values, the samples were made up to these values by the addition of the necessary amount of the raw material. The procedure of heating, grinding and analyzing was continued until the theoretical composition of the compounds was obtained and no free lime was present. The compounds were also examined microscopically to test their purity.

The effects of CaCl_2 upon the four compounds were measured by the rate of hydrolysis. Twelve CaCl_2 solutions ranging in concentrations

¹ Original thesis submitted December, 1934.

from 0.001 per cent to 10.00 per cent were used in this study. In the first part of the experiment a study was made of the rate of hydrolysis when the CaCl_2 solutions were saturated with $\text{Ca}(\text{OH})_2$ while in the latter part of the experiment the CaCl_2 solutions were not saturated with $\text{Ca}(\text{OH})_2$. A duplicate series of samples was run to serve as a check. Also pure $\text{Ca}(\text{OH})_2$ was used in the same amount of each of the CaCl_2 solutions and in boiled distilled water as standards of comparison.

The OH ion concentrations of the resulting solutions were determined at 25° C. by the electrometric method with a saturated calomel half cell, saturated Agar-KCl bridge and a platinum-platinum black electrode. A Leeds and Northrup Type K potentiometer was used.

A comparison of the effects of the various CaCl_2 solutions upon the four compounds was made graphically by plotting the pH as ordinate against time in days as the abscissa. In the case of the solutions saturated with $\text{Ca}(\text{OH})_2$ it was found that the CaCl_2 solutions with a concentration less than 0.35 per cent accelerated the rate of hydrolysis of $3\text{CaO} \cdot \text{Al}_2\text{O}_3$ and $3\text{CaO} \cdot \text{SiO}_2$ but retarded the hydrolysis of $\beta 2\text{CaO} \cdot \text{SiO}_2$ and $\gamma 2\text{CaO} \cdot \text{SiO}_2$. The CaCl_2 solutions more concentrated than 0.35 per cent retarded the rate of hydrolysis of all four compounds studied.

Tricalcium aluminate differs from the silicates in its reaction with CaCl_2 . In the hydrolysis a maximum pH is attained after which there is a decrease in this value. This can be explained by the fact that $3\text{CaO} \cdot \text{Al}_2\text{O}_3$ reacts with CaCl_2 to form double salts such as $3\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot \text{CaCl}_2 \cdot \text{XH}_2\text{O}$.

The data show that as the concentration of the CaCl_2 solutions increases the pH of the $\text{Ca}(\text{OH})_2$ solutions is lowered. An explanation of this is that CaCl_2 reacts with $\text{Ca}(\text{OH})_2$ to give basic salts such as $\text{CaO} \cdot \text{CaCl}_2 \cdot \text{XH}_2\text{O}$. Thus the formation of these soluble, non-ionized salts causes a lowering of the pH.

The second part of the experiment dealing with CaCl_2 solutions not saturated with $\text{Ca}(\text{OH})_2$ shows that the CaCl_2 solutions accelerate the hydrolysis only of $3\text{CaO} \cdot \text{SiO}_2$. The hydrolysis of the $3\text{CaO} \cdot \text{Al}_2\text{O}_3$, $\beta 2\text{CaO} \cdot \text{SiO}_2$ and $\gamma 2\text{CaO} \cdot \text{SiO}_2$ is retarded by all concentrations of CaCl_2 solutions.

It is a well known fact that when a small amount of CaCl_2 is used as an admixture in cement, it accelerates the setting process, and the final strength of the cement is increased. The normal setting process, according to the colloidal theory of cement, begins with the coagulation of the colloidal silicates and aluminates present. A certain Ca ion concentration is necessary before this process takes place. The catalytic effect of the CaCl_2 on cement is to increase the rate of hydrolysis of the compounds in the clinker which results in the rapid attainment of the Ca ion concentration necessary for the setting process. The transformation of these coagulated silicates and aluminates into crystalline bodies gives the strength to the cement.

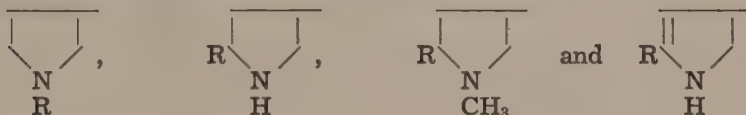
THE IONIZATION CONSTANTS OF SOME SECONDARY AMINES IN METHANOL¹

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Ionization constants have been used as a measure of the relative electronegativity of organic radicals when the latter are linked to identical polar groupings and the ionizations occur in a common solvent. By this means the orders of electronegativity of the organic radicals substituted in the series RNH_2 , RCH_2COOH , RHgX ,



have all been found to be alike².

Most of the work on ionization has been done in water and it seems that its effect on equilibrium reactions has not always been recognized. Some recent investigators³, however, have emphasized the possible disturbing effect of the solvent on the values of equilibrium constants. Other investigators, Goodhue and Hixon⁴ and Goldschmidt and co-workers⁵, have shown that the variations in the relative basicities of a series of compounds are approximately the same in different solvents.

Bronsted's⁶ interpretation of the role of the solvent in dissociation has thus been substantiated to some degree, but more data are necessary for the proper elucidation of the problem.

In this work the ionization constants of several series of secondary amines and a series of α -substituted N-methylpyrrolidines have been measured in methanol to ascertain whether the order of polarity of the radicals substituted coincides with that for the various series mentioned above and also to learn more about the effect of the solvent on the electrical properties of organic compounds.

The secondary amines are represented by the RNHCH_3 , RNHC_6H_5 , and $(\text{R})_2\text{NH}$ series which contain the radicals methyl, ϵ -butyl, β -hydroxyethyl, benzyl, p-anisyl, p-tolyl, phenyl and α -naphthyl.

¹ Original thesis submitted December, 1934.

² Hixon and Johns, *J. Am. Chem. Soc.*, **49**:1786, 1789 (1927); Johns, Peterson and Hixon, *J. Phys. Chem.*, **34**:2218 (1930); Craig and Hixon, *J. Am. Chem. Soc.*, **53**:4368 (1931); Craig, *ibid.*, **55**:2543-50 (1933); Starr, Bulbrook and Hixon, *ibid.*, **54**:3971 (1932).

³ Conant, *Ind. Eng. Chem.*, **24**:466-472 (1932).

⁴ Goodhue and Hixon, *J. Am. Chem. Soc.*, **56**:1329 (1934).

⁵ Goldschmidt and Mathieson, *Z. physik. Chem.*, **119**:439 (1926).

⁶ Bronsted, *Chem. Rev.*, **5**:292 (1928).

The α -substituted N-methylpyrrolidines contain the radicals methyl, ethyl, n-propyl, n-butyl, p-anisyl, phenyl, p-chlorophenyl and β -pyridyl.

The order of relative electronegativity found for several of the radicals substituted in the RNHC_6H_5 and $(\text{R})_2\text{NH}$ series has been compared with that obtained from the dissociation data of Goldschmidt and Bader⁷ for a group of radicals in the α -diaryl substituted β -benzoyltetra-
zanes, $[(\text{R})_2\text{N-NCOC}_6\text{H}_5]_2$.

EXPERIMENTAL

PREPARATION OF COMPOUNDS

Absolute methyl alcohol was prepared by the method of Hartley and Raikes⁸.

The following compounds were obtained from the Eastman Kodak Company and were purified before being used: methyl-aniline, methyl-p-toluidine, phenyl- α -naphthylamine, methyl- α -naphthylamine, diphenylamine, benzylaniline, di- μ -butylamine, dibenzylamine and di-(β -hydroxyethyl)-amine.

The method of Goldberg⁹ was used with some modifications for the synthesis of p,p'-dianisylamine, p,p'-ditolylamine, p-methoxydiphenylamine and p-methyldiphenylamine.

β -Hydroxyethyl-aniline, n-butylmethylamine, β -hydroxyethylmethylamine and methyl-p-anisidine have been prepared from directions in the literature.¹⁰

The criteria of purity for the compounds were boiling points and melting points; neutralization equivalents were also used where it was possible to obtain them.

In the series of compounds used, all purified liquids were immediately sealed in weighed ampules. All crystalline compounds, after purification, were dried in vacuo over calcium chloride for a day and then kept in a vacuum desiccator over phosphoric anhydride.

MEASUREMENT OF IONIZATION CONSTANTS

The ionization constants were measured by means of a hydrogen electrode and a calomel half-cell containing 0.1 formal sodium chloride in methanol. Measurements were made on two solutions of each compound in an air thermostat at $25^\circ \pm 0.1^\circ$ and each reading was checked with a duplicate hydrogen electrode.

The amines were half neutralized with a standard methanol hydrogen chloride solution which was always used within three hours after preparation. The concentrations of all solutions of the amines were determined accurately and were about 0.05 molal.

Two methanol calomel half-cells of e. m. f. 0.0449 volt and 0.0437 volt referred to the silver-silver chloride were used.

⁷ Goldschmidt and Bader, *Ann.*, 473:137 (1929).

⁸ Hartley and Raikes, *J. Chem. Soc.*, 524 (1925).

⁹ Goldberg, *Ber.*, 40:4541 (1907).

¹⁰ Rindfusz and Harnack, *J. Am. Chem. Soc.*, 42:1725 (1920); Graymore, *J. Chem. Soc.*, 1355 (1932); Dains and co-workers, *J. Am. Chem. Soc.*, 47:1982 (1925); Spaeth and Brunner, 58:522 (1925).

The e. m. f. of the silver-silver chloride electrode referred to the normal hydrogen electrode in methanol was calculated to be 0.0711 volt by Buckley and Hartley¹¹ and was added to the voltage of the calomel half-cell to refer the latter to the normal hydrogen electrode.

The method and technique used and the calculations involved in the determination of the ionization constants has been described by Goodhue and Hixon⁴.

DISCUSSION OF RESULTS

The $pK_{b_{CH_3OH}}$ values ($-\log K_{b_{CH_3OH}}$) of all of the secondary amines investigated give an order of relative electronegativity for the substituted organic radicals which corresponds to that for the radicals in the series previously reported (1).

The ionization constants of the symmetrical secondary amines indicate an order of polarity for the $(R)_2-$ series which is the same as that for the $R-$ series mentioned above.

In general, the dissociation constants of Goldschmidt and Bader's α -diaryl substituted β -benzoyl tetrazanes align themselves in an order which corresponds to that for the similarly substituted diphenylamines.

The curves obtained by plotting $pK_{b_{CH_3OH}}$ values of the various series investigated as ordinates against the electron-sharing ability of the substituted organic radicals as abscissas were found to be parallel to those drawn from the pK_b values of the same compounds in water. The solvent and radical effects may be concluded to be constant for each series.

¹¹ Buckley and Hartley, *Phil. Mag.* [7], 8:320 (1929).

THE FREE ENERGY OF FURFURAL AND SOME OF ITS DERIVATIVES¹

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The present investigation was undertaken to secure information concerning the changes in free energy when d-xylose, furfural and some of the products formed by the hydrogenation of furfural were converted into each other. The substances studied were furfural, furfuryl alcohol, tetrahydrofurfuryl alcohol, pentane 1, 5 diol and d-xylose. The free energies were calculated from the entropy and the heat of formation.

The entropy above 90°K was determined by graphical integration of the area under the curve formed by plotting the molal heat capacity divided by the absolute temperature against the absolute temperature.

Heat capacities were determined from 100°K to 273°K or to 298°K. The determinations were made in two ranges, one from 100°K to 220°K, the other from 190°K to 273°K. In the first range the calorimeter was surrounded by liquid air, and in the second range by solid carbon dioxide. The calorimeter consisted of two coaxial gas tight brass cylinders. The small inner cylinder which contained the sample was suspended inside the larger cylinder by silk threads. A heating coil of 125 cm. of No. 31 constantan wire was wrapped around the inner container. An electrical current passing through the coil raised the temperature of the container and the sample. The voltage drop across the coil was measured by a potentiometer and was kept constant by a variable resistance. The temperature change was measured by a calibrated copper constantan thermoelement connected to a student potentiometer. The pressure of the gas in the space between the inner and outer cylinders was kept below 0.1 micron by a vacuum system composed of a mercury diffusion pump backed by a Hyvac oil pump. The apparatus was calibrated with naphthalene using the data of Southard and Brickwedde.⁴

The entropy below 90°K was determined by substituting the proper values in the equation of Kelly, Parks and Huffman.²

$$\Delta S_{90} = \Delta S^{\circ}_{90} + B \int_0^{90} \frac{C_p dT}{T^2}$$

S is the entropy at 90°K, S°_{90} and C_p° are data characteristic of classes of substances, aliphatic or cyclic. A and B are constants found by substituting experimentally determined values at two temperatures, as 110°K and 120°K, and solving the resulting equations simultaneously.

In the heat capacity determination from 90°K to 273°K, three compounds had a transition temperature. Table 1 gives the values. The entropy change of the fusion was obtained by dividing the heat of fusion by the absolute temperature.

¹ Original thesis submitted December, 1934.

TABLE 1.

| Compound | Transition temperature | ΔH of fusion | ΔS of fusion |
|--------------------|------------------------|----------------------|----------------------|
| Furfural | 235.1 | 3434 | 14.60 |
| Furfuryl alcohol | 253.5 | 3540 | 13.58 |
| Pentane, 1, 5 diol | 248.0 | 3759 | 15.15 |

Table 2 gives the entropy values. The change in entropy in the formation of a compound was found by subtracting the absolute entropy values for the elements in each compound from the molal entropy.

TABLE 2.

| Compound | ΔS 0 to 90 calc. | ΔS 90 to 298.16 | molal entropy 298.16 | ΔS elements | ΔS 298.16 |
|----------------------------|--------------------------------|-------------------------------|----------------------------|------------------------|----------------------|
| Furfural | 12.52 | 24.98 | 52.10 | -109.55 | - 57.45 |
| Furfuryl alcohol | 10.27 | 27.65 | 51.50 | -125.16 | - 73.66 |
| Tetrahydrofurfuryl alcohol | 10.81 | 41.61 | 52.42 | -187.62 | -135.20 |
| Pentane 1, 5 diol | 18.76 | 42.91 | 76.82 | -218.85 | -142.03 |
| d-xylose | 7.50 | 26.81 | 34.30 | -224.4 | -190.1 |

The heat of formation of each compound was found by subtracting the sum of the heats of combustion of the elements in the compound from the heat of combustion of the compound. The heats of combustion were determined for furfural and pentane 1, 5 diol by burning them in an Emerson fuel calorimeter. The calorimeter was standardized by benzoic acid obtained from the Bureau of Standards. The heat of combustion at constant volume was found to be 558.3 kilogram calories for furfural and 752.3 kilogram calories for pentane 1, 5 diol. The equation used for the determination of the free energy change is:

$$\Delta F = \Delta H - T\Delta S$$

ΔF is the free energy change, ΔH is the heat of formation, ΔS is the entropy change and T the absolute temperature.

These data are given in table 3.

TABLE 3.

| Compound | ΔH Cp combustion kilogram cals. | $\Sigma \Delta H$ elements | ΔH formation calories | $-T\Delta S =$ cals. | ΔF cals. |
|----------------------------|--|-------------------------------|-------------------------------------|-------------------------|---------------------|
| Furfural | 560.3 (3) | 607.8 | - 47,520 | 15,536 | - 31,984 |
| Furfuryl alcohol | 609.0 (3) | 676.1 | - 67,130 | 21,965 | - 45,165 |
| Tetrahydrofurfuryl alcohol | 709.5 (3) | 812.8 | -103,250 | 40,320 | - 62,930 |
| Pentane 1, 5 diol | 754.5 (3) | 857.1 | -105,060 | 42,345 | - 62,715 |
| d-xylose | 561.0 (1) | 796.8 | -235,750 | 57,000 | -178,750 |

From the free energy values given in table 3, the standard free energy changes at 25° were calculated for the reactions shown in table 4. The

standard free energy is the change in free energy when one mole of product is formed at 25°, from reactants and products at the standard free energy state. A pure liquid or solid is in the standard free energy state. Hydrogen gas present at a partial pressure of one atmosphere is in its standard state. The free energy change was found by subtracting the free energy of the reactants from the free energy of the products. The free energy value of water is -56,560. Table 4 gives the standard free energy of the reactions indicated.

TABLE 4.

| Reactant | Product | ΔF_{298} |
|------------------------------------|-------------------------------|------------------|
| d-xylose | furfural + 3 H ₂ O | -22,914 |
| Furfural + H ₂ | furfuryl alcohol | -13,181 |
| Furfuryl alcohol + 2H ₂ | tetrahydrofurfuryl alc. | -17,755 |
| Furfural + 8H ₂ | pentane 1, 5 diol | -31,731 |

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PHYSIOLOGICAL STUDIES ON RHIZOBIUM

IV. UTILIZATION OF CARBONACEOUS MATERIALS¹

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Although extensive experiments have been conducted with the legume root nodule bacteria, no acceptable theory has been advanced to explain the specificity of the organisms in nodule production. Different species may show similar morphological and cultural characteristics, and yet be unable to bring about nodulation upon any plants except the particular hosts.

It has been shown that the grouping of legume bacteria on the basis of cross-inoculation is similar to that indicated by serological reactions in cross-agglutination. The suggestion has also been made that certain materials given off by the roots of leguminous seedlings at a certain stage of development stimulate the formation of nodules. It has been shown further that the different species of rhizobia differ in their ability to utilize carbonaceous materials. In view of these facts it seems probable that studies of carbohydrate dissimilation will yield information of considerable value in the search for an explanation of the specificity of the several species.

The relative utilization of nitrate and ammonium nitrogen by the various organisms may suggest an explanation of the mechanism of nitrogen fixation. A more or less commonly accepted theory of nitrogen fixation is that the phenomenon occurs through the reduction of elemental nitrogen.

Inasmuch as the organisms of this group grow aerobically, using molecular oxygen chiefly as a hydrogen acceptor, the amount of oxygen consumed should provide an accurate index of the extent of carbohydrate utilization.

The investigations reported here have been concerned with (1) the rate and extent of oxygen consumption by the root nodule organisms grown on various carbonaceous materials, (2) the influence of nitrate and ammonium nitrogen on respiratory activity, and (3) the completeness of oxidation of substrates by the organisms in the respiration process.

Rhizobium meliloti, the alfalfa root nodule organism, and *Rh. japonicum*, the soy bean organism, were used in this work.

The consumption of oxygen was measured by the use of blood gas micromanometers of the Warburg type. The inocula were prepared from cultures of the organisms grown for 3 to 4 days in a glucose-yeast-extract medium. The cells were removed from the medium by centrifuging and were washed in sterile NaCl solution. The basic mineral salt medium was the same throughout the experimental work. A suitable carbonaceous material and either nitrate or ammonium nitrogen were added as indicated in the various experiments. All experiments were conducted in duplicate and the data presented represent averages of closely agreeing duplicates.

¹ Original thesis submitted March, 1935.

The relative suitability of the different carbonaceous materials has been determined from the volume of oxygen consumed by the organisms on the various substrates. The amount of oxygen consumed in a glucose medium was taken as 100 per cent and all other values were calculated in percentage of this figure. The extent of oxidation of the various substrates which could be brought about by these organisms was determined by comparing the volume of oxygen actually consumed with the volume which would be required for complete oxidation of the particular compound to carbon dioxide and water.

The amount of oxygen consumed by the organisms when suspended in a NaCl solution served as a control in all experiments. This endogenous respiration is carried on at the expense of energy materials stored within the cells.

SUMMARY

No significant differences were observed in the rate and extent of oxygen consumption by *Rhizobium meliloti*, the alfalfa root nodule organism, in media containing glucose, mannitol, and sucrose. The total amount of oxygen consumed on each of the three substrates was considerably greater with ammonium nitrogen than with nitrate nitrogen.

Galactose and arabinose were decidedly superior to glucose for utilization by *Rh. meliloti* on both nitrate and ammonium media.

Maltose, lactose, inositol, dulcitol, and sorbitol with either form of nitrogen and raffinose and erythritol with ammonium nitrogen were decidedly inferior to glucose as a source of energy for *Rh. meliloti*. In media containing nitrate nitrogen, raffinose and erythritol permitted approximately the same degree of respiratory activity by this organism as did glucose.

Ammonium nitrogen seemed more suitable for utilization by *Rh. meliloti* than nitrate nitrogen, as measured by the total consumption of oxygen.

Arabinose was distinctly superior to all the other carbonaceous compounds studied, as a source of energy for *Rh. japonicum*, the soy bean root nodule organism.

Glucose, galactose and xylose permitted approximately equal amounts of oxygen consumption by *Rh. japonicum* in media containing nitrate nitrogen. The total volume of oxygen consumed on each of these substrates was slightly less than half of the volume consumed in an arabinose-nitrate medium.

Mannitol, mannose, maltose, glycerol, lactose, sucrose, and erythritol were utilized only slightly or not at all by *Rh. japonicum* under the conditions of this experiment.

Nitrate nitrogen seemed more suitable for utilization by *Rh. japonicum* than ammonium nitrogen, as determined by the total consumption of oxygen.

Glucose and mannitol in concentrations of 1/3, 2/3, and 1 gram per liter were oxidized to from 30 to 34 per cent of completion by *Rh. meliloti* when ammonium nitrogen was present.

Glucose and arabinose in concentrations of 1/3, 2/3, and 1 gram per liter were oxidized to from 36 to 42 per cent of completion by *Rh. japonicum* when nitrate nitrogen was present in the media.

THE PRODUCTION OF BUTYL AND ISOPROPYL ALCOHOLS BY FERMENTATIVE PROCESSES¹

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Glucose is fermented by *Clostridium butylicum* (Beijerinck) Donker with the production of 27 per cent of butyl alcohol and seven per cent of isopropyl alcohol. Carbon dioxide and hydrogen are always formed, and butyric and acetic acids are produced in quantities which vary with changes in environmental conditions.

Cl. butylicum is unable to hydrolyze native proteins, and good growth is obtained only in the presence of partially hydrolyzed proteins such as peptone and the nitrogenous substances in malt sprouts and corn steep water. Bacterial growth and the quantities of glucose fermented are roughly proportional to the degree of hydrolysis of the nitrogenous substrates. Four grams of glucose can be fermented in the presence of 0.24 gm. of peptone nitrogen, of which 23.0 per cent is in the amino acid form; whereas 6.0 gm. of glucose can be fermented in the presence of 0.24 gm. of hydrolyzed peptone nitrogen (85 per cent in the amino acid form). Gelatin, casein, zein and crude corn gluten gave similar results. Malt sprouts and corn steep water serve efficiently as sources of nitrogen.

None of the amino acids is essential for growth. Fermentations were successfully carried out in the presence of the monoamino acids which had been extracted from casein with butyl alcohol. Alanine and leucine, glutamic acid and tyrosine, leucine alone and alanine alone support fermentation, but the results are variable and cannot always be duplicated. The organisms sporulate more freely if tryptophane is present, but the yields of alcohols are not increased.

The work of Reilly (1920) and his associates, of Speakman (1920) and of Donker (1926) with *Cl. acetobutylicum* established the following chemical relationships. Acetic and butyric acids appear early in the fermentation. The acetic acid is converted into acetone and the butyric acid is reduced to butyl alcohol. In the presence of calcium carbonate no acetone or alcohols are produced, the acids accumulating as the calcium salts. In the latter case two moles of carbon dioxide are formed for each mole of glucose fermented and the hydrogen obtained is the sum of twice the moles of sugar fermented plus the moles of acetic acid produced. *Cl. butylicum* differs from *Cl. acetobutylicum* in that the acetone formed is reduced to isopropyl alcohol; there is no accumulation of acids in the early stages of fermentation, and alcohols are produced in the presence of calcium carbonate. If the medium is buffered above pH 6.3 the salts of acetic and butyric acids are formed.

If acetic acid is added to fermenting glucose it is converted into isopropyl alcohol, but its presence in the medium changes the course of the glucose fermentation in such manner as to bring about the formation of

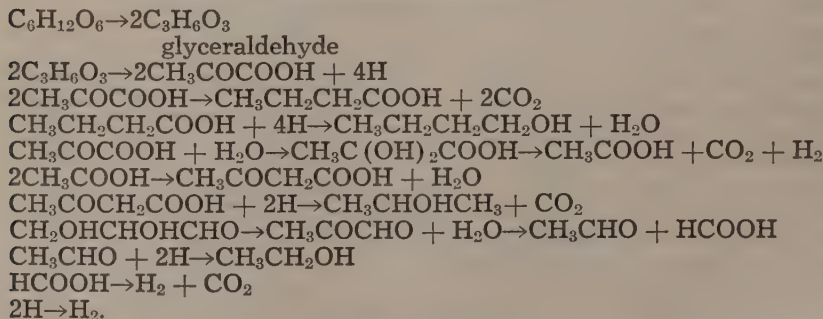
¹ Original thesis submitted December, 1934.

more four-carbon compounds from the glucose. Butyric acid, added to fermenting glucose, is reduced to butyl alcohol, but the course of the glucose fermentation is shifted so that more acetic acid and isopropyl alcohol are formed. The carbon dioxide and hydrogen balances show that acetic acid is not formed from the butyric acid added.

If pyruvic acid is fermented it yields a mixture of acetic and butyric acids and carbon dioxide; if it is added to fermenting glucose there is a corresponding increase in acetic and butyric acids and carbon dioxide (or of their products, isopropyl and butyl alcohols). The exact ratio of acetic to butyric acids produced from the pyruvic acid (in the presence of glucose) cannot be determined because of the shift in equilibrium between the acetic and butyric acids produced from glucose.

The addition of acetaldehyde to fermenting glucose leads to the production of ethyl alcohol and acetylmethylcarbinol. In normal glucose fermentation by *Cl. butylicum*, ethyl alcohol is produced only in small quantities (one to two per cent) and acetylmethylcarbinol has never been detected. Acetaldehyde cannot be isolated from glucose fermentations as it can when glucose is fermented by such organisms as *E. coli*, *B. mace-rans*, *B. acetothyllicum* and others which normally produce ethyl alcohol, acetylmethylcarbinol and 2, 3-buteneglycol. The proposed chemical mechanisms which postulate acetaldehyde as the chief intermediate product in butyl alcohol-acetonic fermentations are based on the latter types of fermentation given above.

The following reactions can account for the chemical transformations in the butyl-isopropyl alcohol fermentation:



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THE EFFECT OF CHEMICAL AND PHYSICAL ENVIRONMENT UPON THE FERMENTATIVE ACTIVITY OF ZYMIN¹

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The investigations here reported were undertaken for the purpose of examining the effect of some environmental factors on the yeast enzyme complex which brings about alcoholic fermentation. Zymin was chosen as the yeast enzyme source. Essentially zymin is yeast from which the acetone and ether soluble fraction has been removed by extraction. This preparation is quite incapable of growth or reproduction, and hence contains no living yeast cells, but contains the enzymes in an active state. Certain phases of the work were repeated with dried yeast.

The influence of the salts NH_4Cl , MgSO_4 , CaCl_2 , KCl and NaCl on the activity of zymin as measured by the rate of CO_2 evolution was investigated. Under the conditions of the experiments there existed an optimum concentration for each salt which increased the rate of CO_2 evolution from a zymin reaction mixture. Concentrations were such that no induction period occurred and the maximum rate was attained during the first thirty minutes. During the 30 to 90 minute period the rate was more constant but gradually decreasing. The salt activation was divided into the effect on the initial, maximum rate, and the effect on the gradually diminishing steady rate. NH_4Cl and MgSO_4 caused a marked increase in both rates; NaCl and KCl increased the initial rate appreciably but had little effect on the steady rate; CaCl_2 increased the steady rate but had little effect on the initial rate. When the two most potent salts, NH_4Cl and MgSO_4 were added together the increase in rate was not equal to the sum of the increases caused by each when added separately. When inorganic phosphate was added to a fermentation mixture the expected increase in the rate of CO_2 evolution occurred. When either NH_4Cl or MgSO_4 was added along with phosphate a higher rate was observed.

Activation of the zymase enzyme complex by salts is contrary to the "salt effect" of Harden and Henley (1921), who concluded that salts have a depressing effect on the enzymes concerned with both esterification of phosphate and liberation of organic phosphate. These authors based their conclusions on experiments made with zymin in the presence of 0.25 and 0.4 M. NaCl and Na_2SO_4 , and these concentrations are much greater than the optimum concentration of any salt used in this investigation.

When a smaller concentration of zymin was used an induction period occurred during which no CO_2 was evolved. All of the salts which increased the rate of CO_2 production shortened the length of this induction period when added to a reaction mixture. The potencies of the cations in shortening the period were in the order $\text{NH}_4^+ > 1/2 \text{ Mg}^{++} > \text{Na}^+ > \text{K}^+ > 1/2 \text{ Ca}^{++} > \text{control}$. This confirms the work of Katagiri and Yama-

¹ Original thesis submitted June, 1935.

gishi (1929), who found almost the same order or potency for shortening the induction period with dried yeast.

The optimum pH for zymín activity during the first 90 minutes was found to be 5.8-6.2, and is therefore somewhat more sensitive to changes in the hydrogen ion concentration than is living yeast, dried yeast, or yeast juice. This is approximately the pH value reported by Mahdihasson (1930) for the interior of the yeast cell.

The rate of CO_2 production by zymín was found to be 34 per cent greater in a 2 per cent glucose solution than in a 12 per cent solution. This is typical of enzymatic reactions, but is a much greater variation than has been reported previously for a yeast enzyme preparation.

The effect of ethanol on the activity of zymín and dried yeast was investigated. The relationship between the relative rate (rate with pure water = 100) and the percentage ethanol added was found to be linear for both yeast preparations. However, a concentration of 13.5 per cent ethanol was necessary to decrease the relative rate of CO_2 production by a dried yeast reaction mixture to 50, whereas 1.8 per cent was sufficient for a zymín reaction mixture. Data of Rahn (1929) indicate that the relation between relative rate and percentage ethanol is also linear for living yeast, and that 6.5 per cent ethanol reduces the relative rate to 50. Addition of inorganic phosphate did not decrease the inactivation of zymín by ethanol. NH_4Cl caused a much larger percentage increase in the rate of CO_2 production in the presence of added ethanol than in its absence, while MgSO_4 brought about the same percentage increase in either case.

Another part of the investigation had to do with the influence of environmental factors on the inorganic phosphate content of zymín and dried yeast fermentation mixtures. It was found that the optimum pH for the disappearance of inorganic phosphate from the medium was 6.2-6.4. This agrees with the value given by Euler and Nordlund (1921) for the synthesis of hexosephosphates by yeast enzymes.

When the zymín concentration was so large that no induction period occurred the inorganic phosphate content reached a minimum value within 30 minutes. NH_4Cl increased the rate of disappearance. However, the normal rate was too great for convenient determination of the effect of other salts. Therefore lower concentrations of zymín were used, and the normal rate of disappearance of phosphate was slower. The salts NH_4Cl , MgSO_4 , KCl and NaCl markedly decreased the time which elapsed before the inorganic phosphate content reached a minimum value. The potencies of the cations were in the order $\text{NH}_4^+ > 1/2 \text{Mg}^{++} > \text{Na}^+ > \text{K}^+ > \text{control} > 1/2 \text{Ca}^{++}$.

Ethanol greatly increased the time necessary for esterification of inorganic phosphate in a zymín reaction mixture, and increased the constant minimum value of the inorganic phosphate content. Ethanol had little influence on the disappearance of inorganic phosphate from a dried yeast fermentation mixture. These results were expected from a comparison of the effect of ethanol on CO_2 production by zymín and dried yeast. In the presence of ethanol the salts NH_4Cl , NaCl and KCl decreased the minimum, constant, inorganic phosphate content in the order $\text{NH}_4^+ > \text{Na}^+ > \text{K}^+$, while MgSO_4 and CaCl_2 slightly increased this value.

On the basis of the results obtained it is suggested that salts activate that part of the zymase enzyme complex which has to do with esterifica-

tion of inorganic phosphate and sugar, and in some cases that part which has to do with the release of organic phosphate. It is further suggested that ethanol seriously interferes with the phosphate esterifying mechanism of zymine, and hence with CO_2 production, because some protective substance is extracted in its preparation, and because of a fundamental change in the physico-chemical system.

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SELECTIVITY OF BORIC ACID MEDIA IN THE COLON GROUP¹

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Investigations were made concerning the effect of concentration of boric acid, nutrients, reaction (pH) and buffer mixtures, together with variation of temperature of incubation on growth and gas production from lactose by members of the colon-aerogenes group of bacteria². As a result of these investigations a medium was developed which may be useful for the selective isolation of members of the genus *Escherichia*.

The medium had the following composition:

| | | |
|---|--------|-------|
| Proteose peptone (Difco) | 10 | grams |
| Lactose | 5 | " |
| K ₂ HPO ₄ (anhydrous) | 12.203 | " |
| KH ₂ PO ₄ | 4.085 | " |
| Boric acid | 3.250 | " |
| Andrade indicator | 10 | cc. |
| Distilled water | 1000 | cc. |

The medium was heated to dissolve the ingredients, tubed in Durham tubes and sterilized at 15 pounds steam pressure for 15 minutes. The inoculum consisted of a 1.5 mm. loopful of 24 hour nutrient broth culture of the test organism.

Of 227 strains of the genus *Escherichia* observed, 220 cultures (96.9 per cent) grew luxuriantly in the medium maintained at 42.5°-43.5° C. for 48 hours whereas among 305 strains of the genus *Aerobacter* only 59 cultures (19.4 per cent) showed visible growth and of 99 strains of the genus *Citrobacter* investigated, 28 cultures (28.3 per cent) showed visible growth in the medium held at 42.5°-43.5° C. (temperature of the medium) for 48 hours.

Of 227 cultures of the genus *Escherichia*, 96.5 per cent (219 strains) produced gas in the medium whereas none of the 305 strains of the genus *Aerobacter* and only 4 strains of the genus *Citrobacter* were able to produce gas when the medium was maintained at 42.5°-43.5° C. for 48 hours.

Throughout the course of the foregoing investigations pure cultures of the colon-aerogenes bacteria were used. Practical application of the above medium for the isolation of members of the genus *Escherichia* and the exclusion of members of the genera *Aerobacter* and *Citrobacter* which might be present in water, milk and other food products, will necessitate studies on modifications of the above boric acid medium which will adapt it to the purpose in view.

Hexamine lactose peptone water (Wilson, 1933) was not found suitable as a medium for the growth of members of the genus *Escherichia*

¹ Original thesis submitted March, 1935.

² Also see communications of Levine (1921) and Levine, Epstein and Vaughn (1934).

and the exclusion or inhibition of strains of the genera *Aerobacter* and *Citrobacter*.

Pure cultures of the genus *Escherichia* grew well in hexamine lactose broth. Of 180 strains of the genus *Escherichia* observed, 175 strains (97.2 per cent) produced visible growth in hexamine lactose broth incubated at 37° C. for 48 hours when light inoculations were made from young agar slant cultures. When heavy inoculations were made visible growth was developed by all of the strains of the genus *Escherichia* studied.

Many strains of the genera *Aerobacter* and *Citrobacter* grew in the presence of hexamine. Of 235 strains of the genus *Aerobacter* observed, 166 cultures (70.6 per cent) produced visible growth in the hexamine medium and 48 strains (81.4 per cent) of 59 cultures of the genus *Citrobacter* produced gas in the hexamine medium. Heavy inoculations from young agar slant cultures did not induce visible growth of those strains of the genera *Aerobacter* and *Citrobacter* which did not produce visible growth in the hexamine broth when light inoculations were used.

Telluric acid lactose peptone bile salt broth (Chalmers, 1934) was not found to be a satisfactory medium for the growth of members of the genus *Escherichia*.

Of 25 strains of the genus *Escherichia* observed only 3 strains were able to grow and produce gas in the telluric acid medium held at 37° C. for 4 days. Among 25 strains of the genus *Citrobacter*, only three cultures were able to grow and produce gas and none of the 25 strains of the genus *Aerobacter* produced visible turbidity in the telluric acid broth maintained at 37° C. for 4 days.

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THE OXIDATION, REDUCTION, AND HYDROLYSES OF SILK FIBROIN¹

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The literature of the effects of chemical treatments which silk undergoes in fabrication and use, including oxidation, reduction, and degradation by acid, alkali, salt, and steam, has been reviewed. The work reported by this thesis is a quantitative study of the effect of oxidizing and reducing agents, concentrated acid and alkali, salt, and steam upon silk fibroin and the effect of oxidizing and reducing agents, dilute and concentrated acid and alkali, and steam upon wild silk fibroin as measured by the weight, nitrogen, and mechanical failure of the residual fibroin.

Silk and wild silk fibroin have been oxidized for ten hours at 40° C. by 2.1800 N and 4.3601 N hydrogen peroxide, 0.2350 N hypochlorous acid, and 0.0710 N potassium permanganate in neutral solution. The degradation effected increased with increasing concentration, fibroin being more sensitive to oxidative degradation than wild silk fibroin. At a given concentration, degradation by potassium permanganate has been shown to be a linear function of the volume of this oxidizing agent.

Silk and wild silk fibroin have been treated for ten hours at 40°C. with 0.1844 N sodium hydrosulfite in acetic acid solution. The slightly different values obtained for the fibroin after this treatment are within the limits of experimental error.

Wild silk fibroin has been subjected to hydrolysis for ten hours at 40°C. by 0.4977 N, 1.0263 N, 1.2264 N, and 2.1216 N hydrochloric acid, and for one hour at 100°C. by 0.0304 N, 0.1019 N, and 0.1950 N hydrochloric acid. The resulting degradation has been shown to increase with increasing concentration of acid, the weight and nitrogen of the residual fibroin being logarithmic functions, $y = ax^b$, of the concentration of the acid. Acid degradation of silk and wild silk fibroin tends to leave a residue richer in nitrogen than the original fibroin. Wild silk has been shown much more stable than silk to degradation by acid. An increase in temperature has been shown to increase this degradation. Silk and wild silk fibroin have been treated with hydrochloric acid, sp. gr. 1.145, at 15°C. for thirty seconds. Silk fibroin was much shrunk and gelatinized, although the appearance of wild silk was little affected.

Wild silk was subjected to 0.3256 N, 0.4169 N, 0.5641 N, and 0.9863 N sodium hydroxide for ten hours at 25°C., to 0.2197 N, 0.5641 N, and 0.9863 N sodium hydroxide for ten hours at 40°C. and to 0.0452 N and 0.0969 N sodium hydroxide for one hour at 100°C. Degradation of wild silk by dilute alkali has been shown to increase with increasing concentration of hydrolytic agent, the weight and nitrogen of the residual fibroin being logarithmic functions, $y = ax^b$, of the concentration. Increasing temperature has been shown to increase this degradation. Wild silk has been

¹ Original thesis submitted June, 1935.

shown to resist decomposition by alkali better than silk fibroin. Unlike the alkaline degradation of silk, alkaline degradation of wild silk tends to leave a residue richer in nitrogen than the original fibroin or the residue from acid degradation. Silk and wild silk fibroin have been treated with acid sodium hydroxide, sp. gr. 1.410, at 15°C. for five minutes. Fibroin became transparent during the period of treatment, but its original appearance was restored upon washing. Wild silk fibroin has been shown more resistant than silk fibroin to the action of concentrated alkali.

Silk fibroin subjected to 0.4977 *N* sodium chloride at 25°C. and 40°C. for ten hours has been shown unchanged. Silk fibroin treated for ten hours at 40°C. with 0.1504 *N*, 0.2495 *N*, 0.3463 *N*, and 0.4977 *N* sodium chloride and dried without rinsing for a year has been shown unchanged. Treatment for one hour at 100°C. with 0.0604 *N* and 0.7149 *N* sodium chloride caused no appreciable loss in weight, nitrogen, or mechanical performance.

Fabrics of silk and wild silk fibroin have been steamed for one hour at 15, 30, 45, 60, and 75 pounds pressure, and for one-half, one, two, three and five hours at 60 pounds pressure. The degradation has been shown to increase with increasing time or pressure. Wild silk fibroin has been shown much more sensitive than silk fibroin to degradation by steam.

Mechanical failure of silk and wild silk upon treatment with oxidizing agents, acid, alkali, or steam, has been shown more rapid than loss in weight or nitrogen, indicating a breakdown of fibrous structure preceding formation of soluble decomposition products.

STUDIES ON THE ACTION OF SUBLETHAL PERCENTAGES OF ILLUMINATING GAS¹

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The purpose of this investigation was to study the effects of breathing a sublethal percentage of illuminating gas administered for about an hour daily. The experimental animal was the albino rat (*Mus norvegicus albinus*). Special study of the effects of the experimental treatment on reproductive activity was made.

The gas mixture circulating through the animal chamber of the respiratory apparatus in which the rats were kept during the experimental period contained 1.43 per cent illuminating gas. As the illuminating gas used contained 23.5 ± 0.086 per cent carbon monoxide, the rats in the experimental chamber breathed air containing about 0.34 per cent carbon monoxide.

Certain changes in blood constituents were noted during the experiment:

Immediately following the experimental procedure, the blood of the rats was found to be from 60 to 70 per cent saturated with carbon monoxide. This saturation dropped to 30 per cent at the end of a half hour recovery period in air. It dropped to 20 per cent at the end of an hour, and to 15 per cent at the end of an hour and a half. Twenty-four hours later the blood of the rats contained from zero to five per cent carbon monoxide. This rapid rate of dissociation of the carbon monoxide hemoglobin may be explained on the basis of the comparatively low affinity of rat hemoglobin for carbon monoxide as compared to that for oxygen (1).

The animals breathing the 1.43 per cent illuminating gas mixture showed an increased hemoglobin percentage and red cell volume percentage. The average hemoglobin of all the rats subjected to the experimental procedure was 16.6 ± 0.43 grams per cent at 150 days. The average hemoglobin of the control rats at the same age was 14 ± 0.07 grams per cent. The highest hemoglobin value found was 25.25 grams per cent. This was in a female rat. The highest value found among the male rats was 21 grams per cent of hemoglobin. Among a small group of rats with hemoglobins above normal in response to the experimental procedure, simultaneous hemoglobin and red cell volume determinations showed the average hemoglobin to be 19.9 grams per 100 cubic centimeters of blood, and the average red cell volume percentage to be 75. The color volume index obtained from these data showed no change from the normal.

Animals subjected to the experimental procedure over a very long interval showed a slightly increased fragility of the red blood cells.

The experimental male rats weighed significantly less than normal. Comparisons showed a mean difference of 16 ± 2.5 grams body weight at 50 days, 56.7 ± 2.7 grams at 100 days, and 24 ± 2.2 grams at 150 days of

¹ Original thesis submitted December, 1934.

age. The gassed female rats weighed less than normal at 150 days of age, but not earlier.

The experimental procedure as a whole affected the male rats more seriously than it did the female rats: female rats survived under treatment which killed males. There is some evidence that this is true also of human beings (2).

The sex difference in response to the experimental procedure is exemplified further by the effect upon the reproductive activity of the males and of the females:

The sexual cycle was not interrupted in the female rats. Histological examination of the ovaries showed ovulation and corpora lutea formation to be taking place. Oestrus determinations made showed the oestrus cycle to be increased in length significantly, but not highly so. In one group of rats studied the length of the cycles increased from 4.74 ± 0.14 days to 5.56 ± 0.2 days. The value of the mean difference in this group was 0.82 ± 0.23 .

Female rats which had been subjected to the experimental procedure for as long as four months gave birth to young. These young, with one exception, died within a few days of birth. The exception was the one survivor of a litter of ten borne by a female which had been gassed for four months. It was the only rat born of parents, either of which had been gassed, that survived to weaning age.

As the duration of the experimental period became more extended, the condition of the female rats grew progressively worse, and it was found that female rats gassed for nine months did not bear litters.

Table 1 summarizes the history of the litters born to the gassed female rats. The male parent rats were untreated controls.

TABLE 1.

Showing the mortality of the young born to gassed female rats

| Mother | Litter size | Number alive at end of | | | Age (days) of last survivor |
|--------|-------------|--|----------|----------|-----------------------------|
| | | 2 hours | 24 hours | 48 hours | |
| ♀ 1 | 6 | 0 | 0 | 0 | 0 |
| ♀ 1 | 4 | 3 | 3 | 1 | 3 |
| ♀ 1 | 4 | 3 | 1 | 1 | 2 |
| ♀ 1 | ? | No data recorded; litter did not survive | | | |
| ♀ 3 | 3 | 3 | 0 | 0 | 1 |
| ♀ 3 | 5 | 5 | 2 | 0 | 2 |
| ♀ 5 | 7 | 4 | 1 | 0 | 2 |
| ♀ 5 | 2? | 0 | 0 | 0 | 0 |
| ♀ 7 | 10 | 10 | 8 | 2 | 24* |
| ♀ 9 | 8 | 8 | 1 | 0 | 2 |
| ♀ 11 | 6 | 6 | 2 | 2 | 3 |
| ♀ 13 | 1 | 1 | 0 | 0 | 1 |
| ♀ 13 | | Pregnant when killed; five foeti found. | | | |

* Lone surviving rat killed at weaning age; its last surviving litter mate died at 17 days.

In certain of the cases, the young born were immature, and grossly abnormal. The statement is made in the Journal of the American Medical Association (3) that illuminating gas poisoning may cause abortion.

Male rats lost the ability to procreate in a much shorter interval of experimental procedure. During the entire series of experiments, in which accurate records were kept of twelve males subjected to daily exposures to 1.43 per cent illuminating gas, only two males gave rise to young. None of these offspring survived.

One male rat, gassed a total period of two to three hours only, impregnated two females, each of which bore a litter which survived only a few days. A second male rat, gassed for a total of twenty-three hours, impregnated a female. The litter was born, but none of the young were seen. The other ten males subjected to the experimental procedure produced no offspring, in spite of being kept continuously with normal females which had borne litters.

In the gassed rats examined, no motile spermatozoa were found, with the exception that very slight motility was observed among the spermatozoa taken from that male which impregnated a female after twenty-three hours of experimental treatment. Weights of the testes of the gassed rats were found to be only one-third normal.

In connection with the results obtained upon the gassed male rats, it is of interest to note comments of McCombs (4) and Rossiter (5) which indicate that carbon monoxide poisoning in men has an effect similar to that observed in the male rats.

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THE PHYSIOLOGY OF THE PROPIONIC ACID BACTERIA¹

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The propionic acid bacteria under anaerobic conditions produce propionic acid, acetic acid, CO₂, succinic acid, a non-reducing material, and under certain conditions lactic acid from glucose. The primary purpose of this investigation was to extend our knowledge of the mechanism of the dissimilation of glucose by the propionic acid bacteria. To accomplish such a purpose it is essential to have not only accurate knowledge of the quantities of end-products of fermentation, but it is also necessary to have information concerning the intermediate compounds which are involved in the steps leading to the final conversion products.

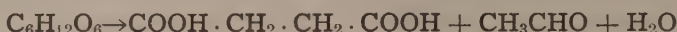
At the time of initiation of this investigation the following facts had been established concerning the identity of intermediate compounds occurring in this dissimilation. Virtanen and Karstrom (1931) had isolated a hexose-monophosphate, and Foote, Fred and Peterson (1930) had reported the production of lactic acid as an end-product of fermentation. Foote et. al. did not state that they considered the lactic acid to be an intermediate product, although Virtanen (1923) (1925) upon theoretical considerations had arrived at this conclusion in the case of glucose dissimilation. Van Niel (1928) as the result of careful work proposed schemes to account for the dissimilation of lactic acid and glucose; however, none of the intermediate compounds was identified or detected. Since the initiation of this investigation Fromageot and Tatum (1933), using the two cultures which produced lactic acid in the experiments of Foote, et. al., have demonstrated the formation of lactic acid and included it in their schemes. Pett and Wynne (1933) isolated methylglyoxal.

Intermediate compounds have been isolated or detected in this investigation using the following methods: (a) By demonstrating quantitatively correlated increases and subsequent decreases of certain compounds by analyses made intermittently on fermentations during the course of the dissimilation. (b) By varying the conditions of the fermentation, thus causing an accumulation of compounds previously not found to occur as end-products. (c) By fixing with NaHSO₃, CaSO₃ or dimedon (dimethyl-dihydro-resorcinol) aldehydes and ketones produced intermediately during the dissimilation. These methods have led to observations which support the conclusion that the following compounds occur intermediately in fermentations by the propionic acid bacteria: (1)_a a non-reducing material, (2)_b lactic acid, (3)_c propionaldehyde, (4)_c pyruvic acid, (5)_a acetic acid, (6)_a succinic acid. (Subscript designates the method used in detecting or isolating compound.)

The literature is confusing with regard to the method of formation of succinic acid. Van Niel concluded that succinic acid is not of carbohydrate origin, but is formed from compounds of the yeast extract which

¹ Original thesis submitted December, 1934.

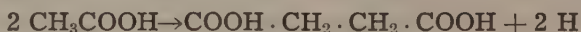
was used as a source of nitrogen in the medium. Virtanen and Foote, et al. report that the succinic acid is of carbohydrate origin and Virtanen (1923) (1925) proposes that it is formed by a 4- and 2- carbon cleavage.



Wood and Werkman (1934_a) pointed out that Virtanen's results can not be considered complete since acetaldehyde or its conversion product is not accounted for.

In order to extend our knowledge regarding these problems and to obtain evidence for use in constructing a scheme of fermentation the products of propionic acid fermentation were determined quantitatively and carbon and oxidation-reduction balances calculated to check the accuracy of the determinations. Fermentations substantially different from those described in the literature were obtained in media containing CaCO_3 buffer. The results varied markedly with the culture used. The ratio of propionic acid to acetic acid varied from 1.78 to 14.67, the ratio of CO_2 to acetic acid from 0.93 to 6.26 and the succinic acid ranged from 4.1 per cent to 18.2 per cent of the carbon of the fermented sugar. The schemes which have been suggested thus far are not adequate to meet the requirements of these data. The results, however, give satisfactory carbon and oxidation-reduction balances, and the purity of the cultures producing the fermentations was established.

Generally the schemes of glucose dissimilation represent the hexose chain as being split into two 3- carbon molecules. The 4- and 2- carbon cleavage of Virtanen is an exception. The 3- carbon scheme requires that every 2- carbon product be accompanied by a 1- carbon compound. The large excess of CO_2 compared to acetic acid which was produced in certain fermentations suggests the occurrence of a 2- carbon intermediate compound which by synthesis is converted to a compound of a higher number of carbon atoms. Succinic acid may originate by such a synthesis from acetic acid.



The established intermediate behavior of acetic acid supports such a proposal. In this case for each molecule of succinic acid produced two molecules of CO_2 or some other 1- carbon compound will be formed. In certain fermentations the established quantities are greater and in other cases less than the CO_2 required by this hypothesis. The occurrence of CO_2 in quantities greater than can be accounted for by the formation of succinic acid through a synthesis from acetic acid indicates there is another source of CO_2 . This CO_2 may originate in the formation of propionic acid; a decarboxylation of succinic acid would satisfy such a scheme. The proposal is supported by the fact that succinic acid has been found to act as an intermediate compound. The yields of CO_2 which are less than the calculated amounts when succinic acid is assumed to be formed by a synthesis may be accounted for by incorporation of the 4- and 2- carbon cleavage of Virtanen, thus forming succinic acid without directly involving a 1- carbon compound.

A scheme of glucose dissimilation based upon the foregoing proposals has been formulated. It includes the following conversions: (1) the

4- and 2- carbon cleavage of the glucose molecule as suggested by Virtanen, (2) the role of pyruvic as suggested by Van Niel and substantiated by Wood and Werkman (1934_b), (3) the intermediate occurrence of propionaldehyde, (Wood and Werkman, 1934_c), (4) a non-reducing compound, (5) the synthesis of succinic acid from acetic acid and (6) the decomposition of succinic acid to CO₂ and propionic acid. Evidence is presented, also, in support of the intermediate occurrence of methylglyoxal, alpha-hydroxy-propionaldehyde, and lactic acid. The scheme is substantiated by the fact that it utilizes intermediate products which with the exception of alpha-hydroxy-propionaldehyde have been established in the propionic acid fermentation. Substitution into the scheme of observed values of end-products also proves that it meets the requirements of the quantitative data.

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STUDIES ON SOME TRICHOMONAD FLAGELLATES FROM BIRDS WITH DESCRIPTIONS OF FIVE NEW SPECIES AND TWO NEW VARIETIES¹

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Many species of the genus *Trichomonas* have been described, but comparatively few have been recorded from avian hosts. During the past three years the author has had opportunity to examine many birds for intestinal flagellates. In the present paper appear descriptions of five new species and two new varieties, and new host records for three other species.

The parasites were studied both while living and in preparations that were fixed in Schaudinn's fluid and stained with Heidenhain's iron-hematoxylin. Measurements were made entirely from stained specimens; the width was taken at the widest point, the length along the axis extending from the anterior tip of the body to the distal tip of the axostyle.

Trichomonas (Trichomonas) fulicae n. sp.

(Plate I, figs. 8, 9)

This flagellate was present in the caeca of one of the three American Coots *Fulica americana americana* (Gmelin) (type host) examined by the writer. Pyriform shapes predominated in living material, but many heavily vacuolated individuals were decidedly spherical. Stained specimens are pyriform and slightly curved. The mean dimensions for 100 individuals are $4.7 \times 8.1\mu$; range in width, $4-6\mu$; range in length, $6-10\mu$. These measurements are given in greater detail in table 1.

A broad crescentic cytostome is observed in many specimens. Four free flagella, $6-8\mu$ long, are directed anteriorly. A fifth flagellum follows along the margin of the undulating membrane and extends free $4-5\mu$ from the posterior end of the animal. The undulating membrane is narrow and not easily seen on stained material.

The blepharoplast appears to be composed of a single, large, round granule situated at the extreme anterior end of the body. The costa or chromatic basal rod is long, rather slender, and strongly siderophilous. The axostyle is inconspicuous and hyaline. It projects $4-5\mu$ from the posterior end of the body and tapers somewhat abruptly to a point. Often there is a long filamentous tip such as Kirby (1931) has described as being typical in the genus *Trichomonas*.

This species has a fibril much smaller in diameter than the costa, which differs in that it does not follow the base of the undulating mem-

¹ Journal paper No. J257 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 329.

brane. It arises from the blepharoplast, curves towards the periphery of the body, passes to the right of the nucleus, and frequently reaches the posterior end of the animal's body. In some cases the distal end appears to be attached to the costa.

Wenrich (1921), in *T. muris*, described a peculiar structure near the nucleus which he considered to be the parabasal body. Kirby (1931) described a filament which is similar to the one seen in *T. fulicae* n. sp. He considered the filament to be different from the parabasal thread or fibril and called it the innominate filament. Wenrich and Emmerson (1933) both figured and described the parabasal apparatus of *T. vaginalis* Donne as having a large parabasal body (chromophobic) with a long parabasal fibril (chromophilic). Crouch (1933), figured such a fibril in *T. wenrichi* from the woodchuck, and called it a chromatic rod. The parabasal body is not preserved in the coot material. This fibril is much like the chromophilic portion of the parabasal apparatus, the parabasal fibril, described by Wenrich, but it is considered in the *Trichomonas* of the coot to be an innominate filament.

The nucleus is prominent and broadly ellipsoidal with an average size of $1.0 \times 2.2 \mu$. A rather large and slightly eccentric karyosome is present. A row of small granules is arranged around the inside of the nuclear wall.

TABLE 1. Correlation table of the length and width of *Trichomonas fulicae* n. sp. from the American Coot

| Width in microns | Length in microns | | | | | | | | | Total |
|------------------|-------------------|-----|----|-----|----|-----|----|-----|----|-------|
| | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 | 10 | |
| 3.5 | 1 | 1 | 4 | 2 | 2 | 1 | | | | 11 |
| 4 | 1 | | 3 | 3 | 6 | 2 | 2 | 5 | | 22 |
| 4.5 | | | 3 | 3 | 2 | 1 | 1 | 1 | | 11 |
| 5 | | 1 | 3 | 6 | 15 | 4 | 5 | 4 | 1 | 39 |
| 5.5 | | | 1 | 5 | 3 | 1 | 5 | 1 | 1 | 17 |
| Total | 2 | 2 | 14 | 19 | 28 | 9 | 13 | 11 | 2 | 100 |

| | Length | Width |
|-------|------------|---------------|
| Mean | 8.1μ | 4.7μ |
| Range | $6-10 \mu$ | $3.5-5.5 \mu$ |

Trichomonas (Trichomonas) coccyzi n. sp.

(Plate I, figs. 10, 11)

A light infection of this flagellate was observed in the large intestine of a Yellow-billed Cuckoo, *Coccyzus americanus americanus* Linnaeus (type host), collected at Ruthven, Iowa. The body form was decidedly crescentic from a side view, and slender spindle-shaped from either a dorsal or ventral view. The mean dimensions for 100 specimens were $4.15 \times 10.78 \mu$; range in width, $3-6 \mu$; range in length, $7.5-13 \mu$. These data may be seen in more detail in correlation table 2.

The cytostome is narrow and elongate. Four free flagella extend forward $8-10 \mu$, two of which are somewhat longer than the other two. A fifth flagellum extends along the outer edge of a rather high undulating membrane, and projects free $4-5 \mu$ at the posterior end.

The blepharoplast appears to be composed of a single large round granule. A slender, strongly staining costa reaches to the distal end of the axostyle. A long innominate filament, much like that in *T. fulicae*, extends the full length of the body, and at times appears to be attached to the distal tip of the costa. The axostyle is hyaline and inconspicuous. It ends in a point at the posterior end of the animal and seldom projects. The upper portion of the cytostome is bordered by the ventral portion of the axostyle. A darkly staining, plate-like structure apparently lines the dorsal surface of the cytostome.

The nucleus is ellipsoidal, measuring $1.5 \times 2.0\mu$, and is located near the blepharoplast. A karyosome has a central position and is surrounded by a narrow, clear halo. A mass of small chromatic granules lies between the halo and the nuclear wall.

TABLE 2. Correlation table of the length and width of *Trichomonas coccyzi* n. sp. from the Yellow-billed Cuckoo

| Width in microns | Length in microns | | | | | | | | | | | | |
|------------------|-------------------|---|-----|---|-----|----|------|----|------|----|------|----|-------|
| | 7.5 | 8 | 8.5 | 9 | 9.5 | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | Total |
| 3 | 1 | | | | | | | | 2 | | | | 3 |
| 3.5 | 1 | | | 2 | 3 | 6 | 2 | 1 | 2 | 2 | | 1 | 20 |
| 4 | | 2 | 1 | 1 | 3 | 14 | 5 | 4 | 3 | 7 | 2 | | 42 |
| 4.5 | | | | 1 | 2 | 5 | 2 | 4 | 5 | | 1 | 3 | 23 |
| 5 | | | | 1 | | 1 | 1 | 2 | | | | | 5 |
| 5.5 | | | | | | 1 | 2 | 2 | | | 1 | | 6 |
| 6 | | | | | | 1 | | | | | | | 1 |
| Total | 2 | 2 | 1 | 5 | 8 | 28 | 12 | 13 | 12 | 9 | 4 | 4 | 100 |

| | Length | Width |
|-------|--------------|-----------|
| Mean | 10.6 μ | 4.0 μ |
| Range | 7.5-13 μ | 3-6 μ |

Trichomonas (Tritrichomonas) beckeri n. sp.

(Plate I, figs. 5, 6, 7)

Great numbers of this flagellate were present in the large intestine of a Yellow-billed Cuckoo, *Coccyzus americanus americanus* Linnaeus (type host), collected at Ruthven, Iowa. The body shape is typically slender, pyriform and slightly curved, although many rather spherical specimens are always present. The mean dimensions for 100 specimens are 5.9μ in length, 3.4μ in width; range in length, 4-8 μ ; range in width, 2.5-4.5 μ . These measurements may be seen in correlation table 3.

A small crescentic cytostome is present, located in the typical position. The three free flagella, 12-20 μ long, are often directed posteriorly and in many cases lie so close to the body that counting is made difficult. The fourth flagellum extends along the margin of an undulating membrane, which is so narrow that it is almost never seen in stained material, and projects free 5-7 μ from the posterior end.

The blepharoplast seems to be composed of a single large granule. The costa is slender and quite indistinguishable, usually reaching to near the point where the axostyle projects from the body. Frequently it is seen as a row of granules rather than as a rod.

The axostyle is hyaline and easily seen. It projects $1.5-2.5\mu$ from the posterior end of the animal. Two periaxilar rings are present.

The nucleus is round, $1-1.5\mu$ in diameter. Occasionally a small central karyosome is observed, but usually the chromatin is seen as a mass of small granules. A group of small siderophilous granules frequently obscures the nucleus. These granules are on the dorsal side of the body. When the animal is viewed from the dorsal or ventral surfaces these granules and the nucleus make the organism appear confusingly like a Hexamita.

This species is named for Dr. E. R. Becker in appreciation of the kindly aid and encouragement given to the writer during his studies of these and other protozoa.

TABLE 3. Correlation table of the length and width of 100 specimens of *Trichomonas beckeri* n. sp. from the Yellow-billed Cuckoo

| Width in microns | Length in microns | | | | | | | | | Total |
|------------------|-------------------|-----|----|-----|----|-----|----|-----|---|-------|
| | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | |
| 2.5 | | | | | 3 | | 1 | | | 4 |
| 3 | 1 | 3 | 5 | 5 | 4 | 5 | 2 | 2 | 2 | 29 |
| 3.5 | 1 | 2 | 9 | 13 | 9 | 4 | 6 | 1 | 2 | 47 |
| 4 | | | 4 | 8 | 2 | 2 | 1 | 2 | | 19 |
| 4.5 | | | | | 1 | | | | | 1 |
| Total | 2 | 5 | 18 | 26 | 19 | 11 | 10 | 5 | 4 | 100 |

| | Length | Width |
|-------|----------|--------------|
| Mean | 5.9μ | 3.4μ |
| Range | $4-8\mu$ | $2.5-4.5\mu$ |

Trichomonas (Tritrichomonas) porzanae n. sp.

(Plate I, figs. 1, 2)

This peculiar flagellate was present in the caeca of a Sora Rail *Porzana carolina* (Linnaeus) (type host), collected at Ruthven, Iowa. The body is broadly spindle-shaped with homogeneously staining cytoplasm. The anterior end is frequently broad and blunt. The mean dimensions for 100 individuals is $4.4 \times 7.9\mu$; range in width, $3.5-6\mu$; range in length, $5.5-11.5\mu$. These data may be seen in more detail in table 4.

The broad crescentic cytostome is often margined on one side by the undulating membrane. Usually the membrane is very high in the first loop, and in this case it seems to form one side of the cytostome. The membrane is very narrow with the exception of the first loop. A flagellum margins the undulating membrane and extends free, $3-5\mu$ at the posterior end of the animal. The three anterior flagella are $8-12\mu$ long, and slender.

The blepharoplast is a single large granule situated at the extreme anterior end of the animal. The costa is slender and indistinct, more often composed of a line of granules that follow the contour of the undulating membrane. The axostyle is a slender hyaline rod with feebly staining walls and seldom can be traced into the cytoplasm. It tapers gradually to a point and projects $2-5\mu$ from the body.

The nucleus is large, 2-3 μ in diameter, conspicuous, and round to broadly ellipsoidal in shape. The chromatin material is concentrated into several large granules.

TABLE 4. Correlation table of the length and width of 100 specimens of *Trichomonas porzanae* n. sp. from the Sora Rail

| Width in microns | Length in microns | | | | | | | | | | | | | Total |
|------------------|-------------------|---|-----|----|-----|----|-----|---|-----|----|------|----|------|-------|
| | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 | 10 | 10.5 | 11 | 11.5 | |
| 3.5 | 1 | | 2 | 1 | 3 | 6 | | 1 | | | | | | 14 |
| 4 | 1 | 3 | | 12 | 9 | 2 | | 1 | 2 | 1 | | 2 | | 33 |
| 4.5 | | 2 | 1 | 9 | 6 | 3 | | 1 | 2 | 1 | 1 | | | 26 |
| 5 | | 1 | | 4 | 1 | | | 3 | | 2 | | | | 11 |
| 5.5 | | | 1 | 2 | | 1 | 1 | 2 | 1 | 1 | | 1 | 1 | 11 |
| 6 | | | | | | | 1 | 1 | 1 | 1 | | 1 | | 5 |
| Total | 2 | 6 | 4 | 28 | 19 | 12 | 2 | 9 | 6 | 6 | 1 | 4 | 1 | 100 |

| | Length | Width |
|-------|----------------|-------------|
| Mean | 7.9 μ | 4.4 μ |
| Range | 5.5-11.5 μ | 3.5-6 μ |

Trichomonas (Trichomonas) hegneri n. sp.

(Plate I, figs. 3, 4)

The caecal contents of two European Partridges *Perdix perdix perdix* (Linnaeus) (type host), collected at Ruthven, Iowa, had a light infection of this flagellate. The species was also present in caecal smears from a California Quail *Lophortyx californica californica* Shaw, collected at Bliss, Idaho, and in smears from the Valley Quail kindly loaned to the author by Dr. Robert W. Hegner. It is a small species, curved-pyriform to quite spherical, and somewhat easily confused with *Trichomonas ortyxis* Hegner. The shorter flagella, conspicuous costa, well developed undulating membrane and curved axostyle without periaxilar rings distinguish this species from *T. ortyxis*. The mean dimensions for 100 individuals was 3.9 x 5.5 μ range in width, 3-4.5 μ ; range in length, 4-8 μ . These data may be seen in table 5.

The relatively large cytostome has a darkly staining plate-like structure on the dorsal surface. The four anterior free flagella are 5-7 μ long. The undulating membrane is narrow and extends almost to where the axostyle leaves the body.

The blepharoplast is small and apparently composed of a single granule. The costa is moderately heavy and long, often extending across the axostyle.

The axostyle is narrow, hyaline, and quite inconspicuous. It curves at the anterior end so the ventral side appears to partially form the dorsal portion of the cytostome. It projects free 0.5-1 μ and tapers abruptly to a point. No periaxilar rings could be observed.

The nucleus is 2-2.5 μ in diameter, round to broadly ellipsoidal in shape and rather indistinct. The chromatin material is arranged in a central karyosome, with a ring of smaller granules near the nuclear membrane. There seems to be no definite halo around the karyosome.

This species is named in honor of Dr. Robert W. Hegner.

TABLE 5. Correlation table of the length and width of 100 specimens of *Trichomonas hegneri* n. sp. from the European Partridge

| Width in microns | Length in Microns | | | | | | | | Total |
|------------------|-------------------|-----|----|-----|----|-----|---|-----|-------|
| | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | |
| 3 | 2 | 2 | | 1 | | | | | 5 |
| 3.5 | 2 | 9 | 7 | 7 | 2 | 3 | 3 | | 33 |
| 4 | 1 | 7 | 7 | 13 | 11 | 2 | 4 | | 45 |
| 4.5 | | | | 3 | 7 | 4 | 2 | 1 | 17 |
| Total | 5 | 18 | 14 | 24 | 20 | 9 | 9 | 1 | 100 |

| | Length | Width |
|-------|-----------|------------|
| Mean | 5.5 μ | 3.9 μ |
| Range | 4-8 μ | 3.45 μ |

Trichomonas (*Trichomonas*) *floridanae* Hegner *colini* n. var.

(Plate II, figs. 12, 13)

Examinations of four Bob-white Quail *Colinus virginianus virginianus* (Linnaeus) (type host), two from Ames, one from Leon, and one from Ruthven, Iowa, showed this trichomonad to be a common inhabitant of the caeca. The body is elongate, in general pyriform, and bent with a vague S-shaped curvature. This flagellate is very similar to *T. floridanae* Hegner but more slender, shorter, and with a body curvature not characteristic of the forms in the Valley Quail. The mean dimensions for 100 specimens were 3.7 x 10.4 μ ; range in length, 8-13 μ ; range in width, 2.5-5 μ . (Table 6.)

The cytostome is narrow and crescentic. There are four anterior flagella, of which three are slender and 7-10 μ long, whereas the fourth is of much greater diameter, 20-23 μ long, and is thrown in loops for its entire length. A fifth flagellum margins the undulating membrane, but does not have a free distal end.

The blepharoplast seems to be composed of one large granule. The costa is narrow and usually does not stain heavily as does this structure in *T. floridanae*. The costa is long, and frequently twists around the body to the right as does the undulating membrane.

The axostyle is large, hyaline and conspicuous. It passes to the right of the nucleus, projects 1.5-3 μ at the posterior end, tapers to an abrupt point, and may display a filamentous tip. A broad capitulum is present at the proximal end, within which is a series of endoaxilar granules. Two to three prominent periaxilar rings surround the axostyle immediately before it emerges from the body.

The nucleus is broadly ellipsoidal to round, filled with small chromatin granules. Occasionally a small karyosome is present.

E. A. Allen (1930, 1931) mentioned a flagellate that she found in Bob-white Quail. She has not given it a name. In 1931 she suggested it was like *T. gallinarum*, but that it required a different pH for development in a culture media. It is not possible to determine from her notes whether or not the species described above is the one she has seen, or whether it is *T. gallinarum*, *T. phasiani*, or a still different species.

TABLE 6. Correlation table of the length and width of *Trichomonas floridanae* Hegner colini n. var. from the Bob-white Quail

| Width in microns | Length in microns | | | | | | | | | | | Total |
|---------------------|-------------------|-----|---|-----|----|------|----|------|----|------|----|-------|
| | 8 | 8.5 | 9 | 9.5 | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | |
| 2.5 | | | 1 | | 1 | | | | | | | 2 |
| 3 | | | 2 | 1 | 3 | | 3 | | | | | 9 |
| 3.5 | 1 | | 1 | 9 | 12 | 3 | 8 | 7 | 1 | | | 42 |
| 4 | | 1 | 3 | 3 | 12 | 4 | 7 | 3 | 2 | 1 | 1 | 37 |
| 4.5 | | | | | 1 | 1 | 3 | 1 | 2 | | | 8 |
| 5 | | | | | 1 | | 1 | | | | | 2 |
| Total | 1 | 1 | 7 | 13 | 30 | 8 | 22 | 11 | 5 | 1 | 1 | 100 |

| | Length | Width |
|-------|------------|-------------|
| Mean | 10.4 μ | 3.7 μ |
| Range | 8-13 μ | 2.5-5 μ |

Trichomonas (Trichomonas) floridanae Hegner *perdicis* n. var.

(Plate II, figs. 17, 18)

This flagellate was found in the caeca of two European Partridges *Perdix perdix perdix* (Linnaeus) (type host), collected at Ruthven, Iowa. The morphology of this species varied considerably in the two birds, one of which was collected in the winter and one in the mid-summer. Flagellates in the former host were broadly pyriform from a lateral view with the cytoplasm extending down well towards the end of the axostyle, which was uniformly arched from distal to proximal end. The endoaxilar granules stained very feebly in the *Trichomonas* from this bird. The flagellates from the bird killed in the summer differed in that the cytoplasm did not extend well towards the end of the axostyle, and this structure was much twisted. Occasional specimens of this type were seen in the other bird. The endoaxilar granules in this instance stained intensely. Measurements differed only slightly in the two birds, 4.6 x 10.3 μ in the bird collected in the summer and 5.1 x 11.4 μ in the bird collected in the winter. Table 7 gives more detailed measurements for the *Trichomonas* from the summer bird.

The cytostome is a broad crescentic area, the border of which is decidedly siderophilous. The cytostome of *T. floridanae* is larger and without the strongly chromophilic border. Four anterior flagella arise near the anterior margin of the cytostome, of which three are very slender, 18-20 μ long, and one heavy, 22-27 μ long, and is like a trailing flagellum except that it is thrown into a series of active loops. A fifth flagellum extends along the margin of the high undulating membrane, but does not have a free end.

The blepharoplast seems to be a complex of several granules. The costa is usually apparent as a strongly staining rod, but is also seen as a linear series of small granules.

The axostyle is wide and hyaline. It extends free from the body 3-4 μ at the distal end, and ends in a sharp point. A filamentous tip 0.5-2 μ long is usually evident. Two to four periaxilar rings surround the axostyle near the free end. The proximal end is expanded to a broad capitulum

within which are a series of granules. In undistorted specimens these granules often seem to enter the cytostome, but this appearance is apparently due both to the curvature of the axostyle and to the fact that the granules tend to be located towards the ventral side of the capitulum.

The nucleus is much more indistinct than this same structure in *T. floridanae*. It has a thin nuclear membrane, with many small granules within. Sometimes a small karyosome is visible. The endoaxilar granules obscure the nucleus.

TABLE 7. Correlation table of the length and width of *Trichomonas floridanae* Hegner *perdicis* n. var. from the European Partridge

| Width in microns | Length in microns | | | | | | | | | | | | | |
|------------------|-------------------|---|-----|----|-----|----|------|----|------|----|------|----|------|-------|
| | 7.5 | 8 | 8.5 | 9 | 9.5 | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 | Total |
| 3.5 | 1 | 1 | 1 | 2 | 2 | 2 | | 1 | | 1 | | | | 10 |
| 4 | | 1 | 2 | 5 | 3 | 3 | 1 | 3 | 1 | | 1 | 1 | | 23 |
| 4.5 | | 1 | 1 | 2 | 6 | 6 | 3 | 2 | 2 | 4 | 2 | 2 | 2 | 33 |
| 5 | | 1 | | 3 | 1 | 3 | 2 | 1 | 2 | | | 1 | | 14 |
| 5.5 | | 2 | 2 | 1 | 1 | 1 | 1 | 1 | | 2 | 1 | 1 | | 13 |
| 6 | | 1 | | | 1 | 1 | 1 | | 1 | 2 | | | 1 | 7 |
| Total | 1 | 7 | 6 | 13 | 14 | 16 | 7 | 9 | 7 | 7 | 4 | 5 | 4 | 100 |

| | Length | Width |
|-------|----------------|-------------|
| Mean | 10.3 μ | 4.6 μ |
| Range | 7.5-13.5 μ | 3.5-6 μ |

Trichomonas (Trichomonas) floridanae Hegner (1929)

(Plate II, fig. 14)

Hegner (1929) described this species from the Valley Quail (type host). He described it as having three anterior flagella, and a group of about 20 spherical chromatin granules located along a median line just posterior to the blepharoplast complex. Specimens on the author's slides from the California Quail, *Lophortyx californica californica* (Shaw), showed three slender anterior flagella and one heavy free flagellum directed posteriorly. The three slender anterior flagella are easily seen on a type slide kindly loaned the author by Dr. Robert W. Hegner, but for some reason the heavy free flagellum stained feebly and is extremely difficult to demonstrate. The chromatic granules are endoaxilar granules and due to the bending of the proximal end of the axostyle often appear to end in the cytostome. The measurements of 100 specimens from the author's slides varied from 9-14 μ in length with a mean size of 5.4 x 10.5 μ as compared with the mean size of Hegner's slide of 5.2 x 9.2 μ for the same species.

Trichomonas (Tritrichomonas) ortyxis Hegner (1929)

(Plate II, figs. 15, 16)

Hegner described this species from the Valley Quail (type host). It is one of the smallest species of *Trichomonas* described, having a mean size of 3.3 x 5.4 μ in this host. This species was observed in the two European Partridges, *Perdix perdix perdix* Linnaeus, studied by the author.

It is slightly smaller in this host. No characters other than size could be detected when compared with *T. ortyxis* on a slide loaned the author by Dr. Robert W. Hegner. The mean size for 100 specimens from the European partridge is $2.9 \times 4.3\mu$; range in width, 2.5μ ; range in length, 3.5μ . The flagella are relatively long in this species, being 11-12 μ long.

Trichomonas (Trichomonas) phasiani Travis (1932)

The Bob-white Quail, *Colinus virginianus virginianus* (Linnaeus), were found to have a light infection of this flagellate. The morphology and size is very similar to the individuals found to be present in Ring-necked Pheasants (type host). The mean size for 100 specimens from the pheasant was $3.0 \times 8.5\mu$, whereas this same species in the Bob-white Quail had a mean size of $3.1 \times 8.0\mu$ for 100 individuals. No differences other than the slight difference in size could be detected.

Trichomonas sp.

(Plate II, fig. 19)

Three specimens of a giant flagellate were found on one slide of the smears from the California Quail. No comparable specimens could be found on the other slides from the same bird. Morphologically they were quite similar to the large *Trichomonas* found in the three quail species studied. There were three slender, short flagella, one long trailing flagellum of large diameter, and one flagellum margining the undulating membrane. The latter did not have a free end. The axostyle had a large capitulum with endoaxilar granules that extended more than half way down the axostyle. The nucleus was large and ellipsoidal, and filled with granules about the same size as the endoaxilar granules. The costa protruded from the body at the posterior end in one specimen.

These specimens are also much like the *Trichomonas* species found in Amphibia. Whether this is a normal parasite of the California Quail, an example of a monster such as Becker (1923) observed in the case of *Herpetomonas muscae-domesticae*, or whether they were typically parasites of another host, and the quail acquired them in his food is only a matter of conjecture.

The sizes of the three specimens were $6.1 \times 24.1\mu$, $8.1 \times 22.2\mu$, and $6.1 \times 18.4\mu$.

DISCUSSION

In *T. coccyi* and *T. hegneri* the upper portion of the cytostome is bordered by the ventral portion of the axostyle. A darkly staining plate-like structure apparently lines the dorsal surface of the cytostome. A similar structure was described by Crouch (1933) in *T. wenrichi*. He noted that it arose from the same blepharoplastic granule as did the axostyle. Kirby (1931) described a structure that he thought might be a part of the cytoendoskeleton or related to the axostyle. He described it as a "small bar located on the border of the cytostomal portion of the body," with a filament extending from the bar to the nucleus. He called this structure a parablepharoplastic bar. Wenrich and Emmerson (1933) discussed briefly a similar structure in *T. vaginalis* and followed Kirby's nomenclature, but they considered the plate to be a part of the anterior

end of the axostyle. In the material studied by the author it was not possible to determine whether this was a portion of the axostyle, a thickening of the periplast on the dorsal surface of the cytostome, or whether it was a separate plate between the periplast and the axostyle. Neither Crouch (1933) nor Wenrich and Emmerson (1933) observed a fibril connecting this bar to the nucleus as did Kirby (1931). The author could detect no such connecting fibril in the specimen studied by him. The plate appears to be merely a thickening of the axostyle in the cytostomal region in *T. coccyxi* and *T. hegneri*.

The flagellates found in the California Quail, Bob-white Quail and the European Partridge show striking similarities. The large *Trichomonas* found in each of the three hosts has three slender anterior free flagella, one heavy, long, trailing flagellum and one flagellum that margins the undulating membrane that does not have a free end.

The widely separated areas in which these birds are endemic, and the fact that biological experiments may prove these flagellates to be separate species, in spite of their morphological similarity, have influenced the author to consider the large flagellate of the Bob-white Quail and the European Partridge as varieties of *T. floridanae* rather than separate species. In fact, further studies and experimentation may show that what the writer considers to be *T. phasiani* (type host Ring-necked Pheasant) in the Bob-white Quail, *T. ortyxis* (type host Valley Quail) in the European Partridge, and *T. hegneri* (type host Bob-white Quail) in the California Quail are actually species distinct from those described from their type hosts. At present no morphological characters have been observed that would even indicate their being considered varieties of the above species.

The writer is indebted to Dr. E. R. Becker for his many helpful suggestions and criticisms; to Dr. Paul L. Errington for collecting the Yellow-billed Cuckoo, one European Partridge, and one Bob-white Quail; to Logan J. Bennett for collecting the Sora Rail, and one European Partridge; to F. N. Hamerstrom, Jr., for collecting two of the Bob-white Quails, and to Dr. Robert W. Hegner for the loan of a type slide from the Valley Quail.

EXPLANATION OF PLATE I

x 3000

Figs. 1, 2. *Trichomonas* (*Tritrichomonas*) *porzanae* n. sp.

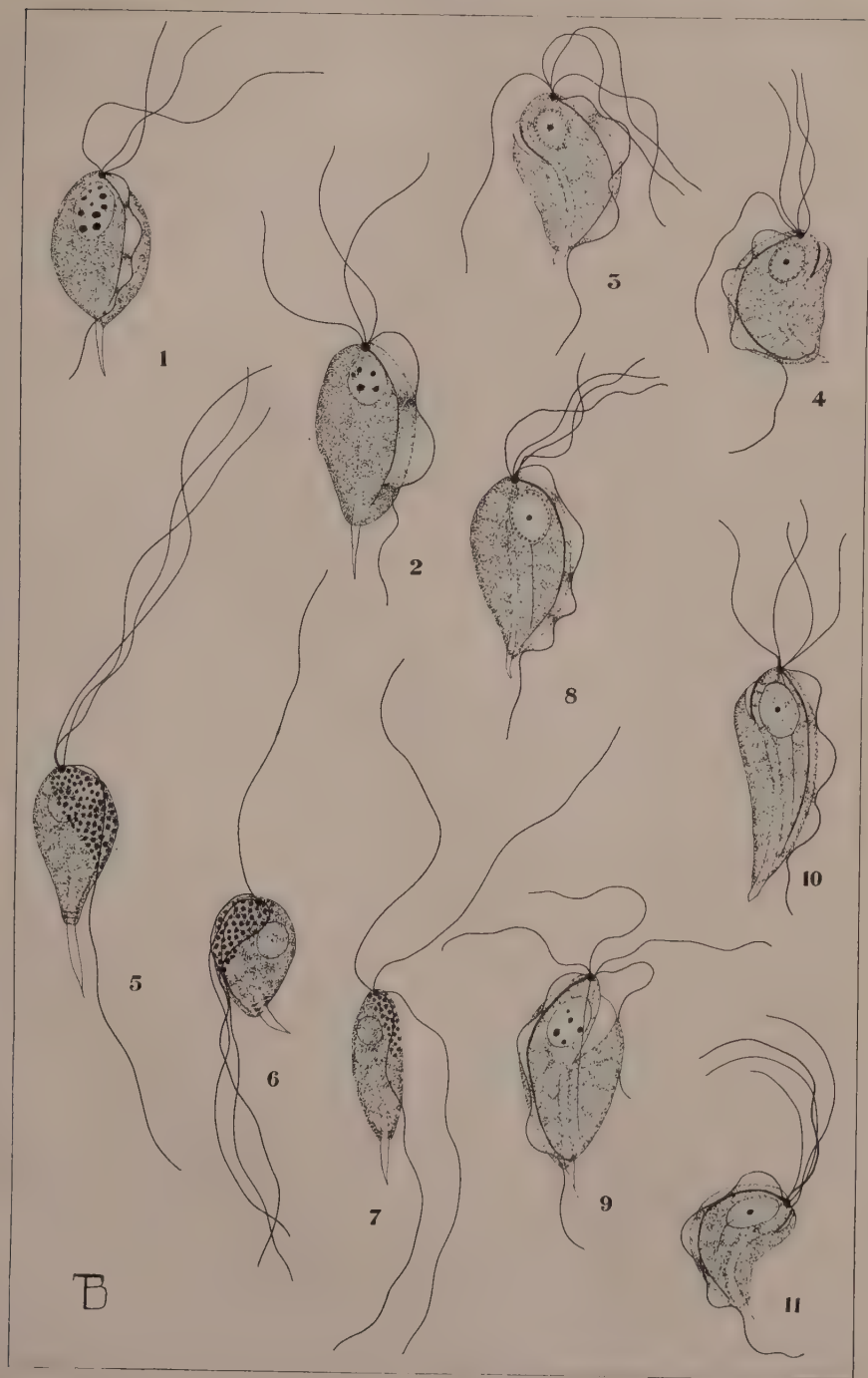
Figs. 3, 4. *Trichomonas* (*Trichomonas*) *hegneri* n. sp.

Figs. 5, 6, 7. *Trichomonas* (*Tritrichomonas*) *beckeri* n. sp.

Figs. 8, 9. *Trichomonas* (*Trichomonas*) *fulcae* n. sp.

Figs. 10, 11. *Trichomonas* (*Trichomonas*) *coccyzi*, n. sp.

PLATE I



EXPLANATION OF PLATE II

x 3000

Figs. 12, 13. *Trichomonas* (*Trichomonas*) *floridanae* Hegner *colini* n. var. from Bob-white Quail.

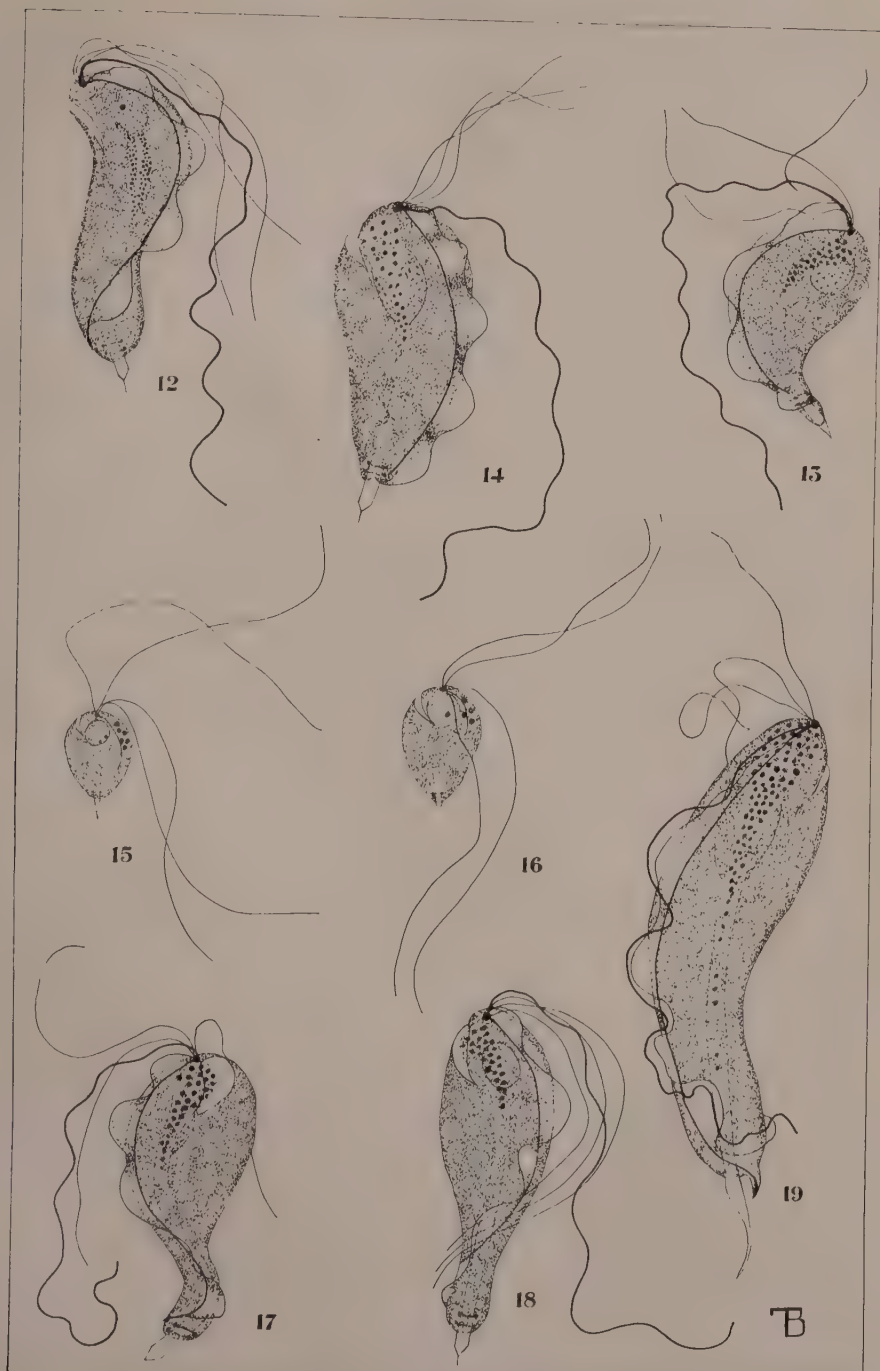
Fig. 14. *Trichomonas* (*Trichomonas*) *floridanae* Hegner from California Quail.

Figs. 15, 16. *Trichomonas* (*Tritrichomonas*) *ortyxis* Hegner from European Partridge.

Figs. 17, 18. *Trichomonas* (*Trichomonas*) *florinanae* Hegner *perdicis* n. var. from European Partridge.

Fig. 19. *Trichomonas* sp. from California Quail.

PLATE II



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THE ACID DEGRADATION OF WOOL KERATIN

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Dilute acid has been shown to increase the dyeing properties of wool (42, 13, 34) and the degradation of wool by alkali (35, 4-6, 24, 22) but to have little immediate effect on its strength below 60°C. (44, 20) and no especially destructive action below 100°C. (36, 29, 15). The rate of shrinkage (39) of wool has been observed to increase with decrease of pH from four (pH = 3.4 to 6.1 has been reported as the isoelectric range of wool keratin 28, 10, 26, 40, 18, 41, 21, 37). Speakman has ascribed the ease of extension of wool fiber in acid to the freeing of long-chain molecules from one another within the micelle (38). Pretreatment with 4 *N* hydrochloric acid for a day has been found to have no permanent effect on the swelling properties of hair (25). Rimington has reported that acid hydrolysis converts the entire sulfur of wool into cystine (32) and Barritt that the maximum methionine sulfur of wool is but three per cent of the total (3). Incipient damage to wool by acid in deliming, sterilizing, carbonizing, fulling, dyeing, or stripping has been found to develop increased resistance to heat (12) but decreased resistance to light (33) and storage (31, 19).

Concentrated acid has long been used at low temperatures as a differential solvent in the recovery of wool from mixtures (7, 9) and it has been reported that wool, though losing an increasing amount of nitrogen in 17.5 to 61 per cent sulfuric acid, loses no nitrogen in 70 per cent sulfuric acid (43). Concentrated hydrochloric acid has been described as splitting hair (25) and 10 *N* hydrochloric acid in five days at 20°C. as softening hair (30).

We have measured the effect on wool of hydrochloric acid in ten hours at 25°C. and of hydrochloric acid and sodium chloride in one hour at 100°C. by the weight, nitrogen, sulfur, and wet breaking strength of the residual wool.

EXPERIMENTAL PROCEDURE

PREPARATION OF WOOL

A wool keratin of 0.3 per cent ash, no sulfate (27), and 0.2 per cent sulfite-yielding sulfur (17) was prepared by boiling plain-woven undyed wool one hour in 100 volumes of water and extracting the dried fabric with anhydrous ether for eighteen hours. The wool was cut into samples for analysis and all but the breaking strength specimens were dried at 105° to 110°C. until successive weighings with tare differed by no more than half a milligram.

ACID DEGRADATION OF WOOL

Six-gram samples of wool were immersed in 125 cc. of water or hydrochloric acid, standardized by precipitation as silver chloride, in a stoppered flask at 25° ± 0.1°C. for ten hours and then washed in water until

the rinse gave no test for chloride. The water-treated wool weighed 99.8 per cent of the untreated, contained 16.60 per cent of nitrogen and 3.93 per cent of sulfur, and had a wet warp breaking strength of nineteen pounds per inch.

Tests at 100°C. for one hour were made similarly with water, hydrochloric acid, or sodium chloride (11) in balloon flasks fitted with water-cooled reflux condensers and heated in a boiling water bath. The 0.7 per cent loss in weight of the wool upon boiling in water for one hour is less than reported losses of 1.5 (16) and 1.25 per cent (22). Titration of blank determinations showed no concentration of the acid or salt during boiling.

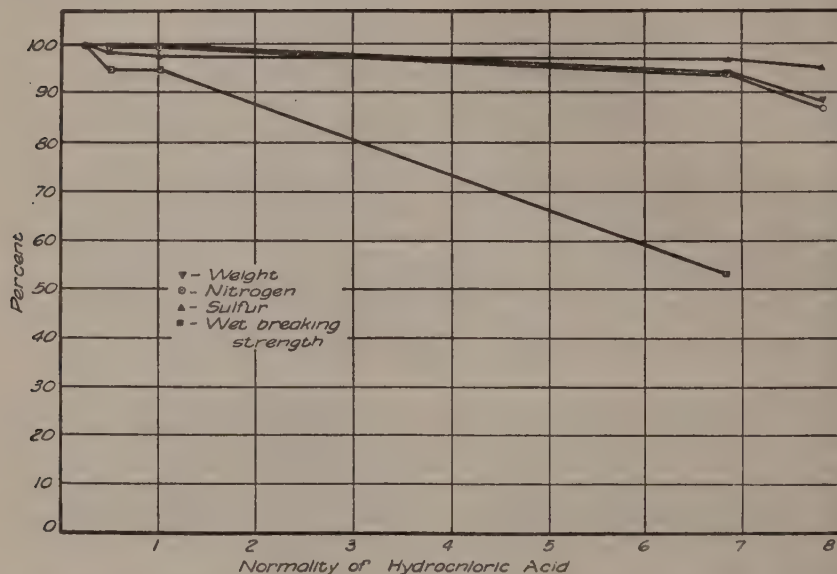


Fig. 1. Effect of acid in ten hours at 25°C. on the weight, nitrogen, sulfur, and wet breaking strength of wool.

TABLE 1. Effect of sodium chloride in one hour at 100°C. on the weight, nitrogen, sulfur, and wet breaking strength of wool

| Sodium chloride | Residual wool | | | |
|-----------------|--------------------|----------|--------|-------------------------------|
| | Weight | Nitrogen | Sulfur | Breaking strength of wet warp |
| normality | percentage of wool | | | pounds per inch |
| 0 | 99.3 | 16.58 | 3.79 | 14 |
| 0.0604 | 99.1 | 16.24 | 3.80 | 16 |
| 0.7049 | 99.1 | 16.47 | 3.78 | 15 |

ANALYSIS OF WOOL

The wet (23) warp breaking strength of ten specimens was determined with a Scott Universal Tester (1). Each value for loss in weight, Kjeldahl-Gunning nitrogen (14), or Benedict-Denis sulfur (8, 2) is the average of four determinations.

The effect of hydrochloric acid in ten hours at 25°C. on the composition and mechanical performance of wool is shown in figure 1. Figure 2

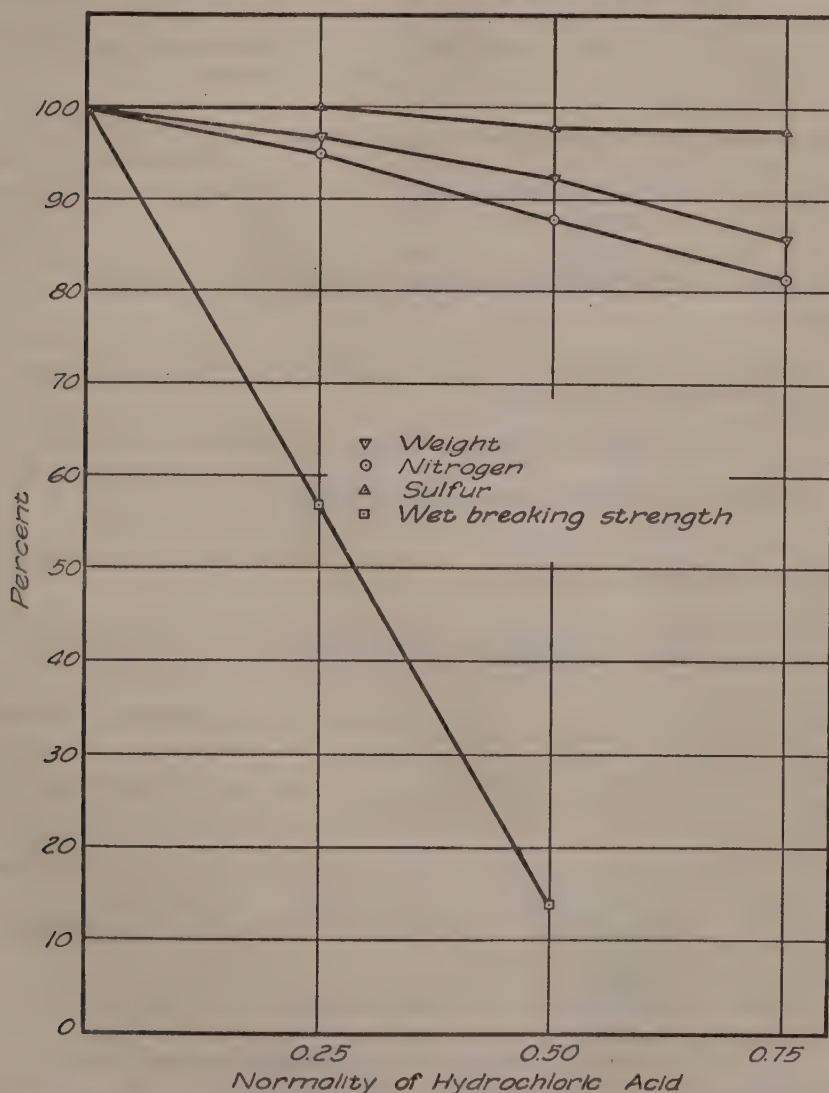


Fig. 2. Effect of acid in one hour at 100°C. on the weight, nitrogen, sulfur, and wet breaking strength of wool.

shows the effect of acid on wool in one hour at 100°C. The residual wool from acid degradation slowly decreased in nitrogen and increased in sulfur with increasing concentration of acid. Table 1 shows a slight solution of nitrogen by 0.06 *N* sodium chloride in one hour at 100°C.

SUMMARY

1. The degradation of wool keratin by 0.25 to 7.87 *N* hydrochloric acid in ten hours at 25°C. and by 0.25 to 0.75 *N* hydrochloric acid and 0.06 to 0.70 *N* sodium chloride in one hour at 100°C. has been followed by the weight, nitrogen, sulfur, and wet breaking strength of the residual keratin.
2. Acid degradation, much greater at 100°C. than at 25°C., has been shown to remove little of the sulfur of wool, to decrease its nitrogen rather similarly to its weight, and to have a sensitive indicator in wet breaking strength.
3. The residual wool has been shown to decrease in nitrogen and increase in sulfur slowly with increasing concentration of acid.

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RELATIVE AROMATICITIES¹

V. RAMAN SPECTRA OF FURANS

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The negative or aromatic character of the furan nucleus has been described as a function of the ease of splitting of mixed organolead compounds²; ease of substitution in the furan nucleus³; tendency for meta-thetical formation of organo-alkali compounds¹; relative reactivity of halides⁴; and of the strength of the carboxylic acids as compared to the corresponding acids of the benzene series⁴. In all of these cases the furan nucleus is characterized by a higher order of reactivity than the benzene nucleus.

Since the Raman frequency is a characteristic which depends upon bond strength (as well as on the mass of the atoms) it was believed that a study of the Raman spectra of some furans might reveal special lines for the C—H and for the C=C linkage or a displacement of the usual frequencies of these linkages which could be correlated with the rather unusual chemical behavior of furan and its derivatives.

We have observed that the line generally characteristic of C=C (1600 cm.⁻¹) is absent in furan itself, although apparently a weak and very diffuse line is present at 1559, which may be due to a C=C linkage. However, furan has a line above 3000 (at about 3158) which has the conventional value for the C=C—H linkage. If the 1559 line which was observed is due to a double bond in furan, there is no discontinuity in the nuclear frequencies in the furan series as has been reported by other workers^{5, 7}. All of the compounds that we have investigated show a frequency of low intensity between 1559 and 1610. We believe that these frequencies are due to the C=C linkages in the furan nucleus.

It has long been known that the three aromatic cycles (furan, thiophene and pyrrole) have resemblances, which in some cases are quite striking in physical, chemical and physiological properties. Furthermore, these heterocycles have highly developed aromatic characteristics. The Raman spectra of some of the furans have been studied by Glockler and Wiener⁵, Matsuno and Han⁶, Bonino, Manzoni-Ansidei and Pratesi⁷,

¹ The preceding paper in this series is in the *J. Am. Chem. Soc.*, **56**, 1123 (1934). A preliminary account of this paper was presented at the Twelfth Midwest Regional Meeting of the American Chemical Society at Kansas City, Missouri, in May, 1934.

² Gilman and Towne, *Rec. trav. chim.*, **51**, 1054 (1932).

³ Gilman and Young, *J. Am. Chem. Soc.*, **56**, 464 (1934).

⁴ Catlin, Doctoral Thesis, Library, Iowa State College, 1934.

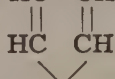
⁵ Glockler and Wiener, *J. Chem. Phys.*, **2**, 47 (1934).

⁶ Matsuno and Han, *Bull. Chem. Soc. Japan*, **9**, 327 (1934).

⁷ Bonino, Manzoni-Ansidei and Pratesi, *Z. physik. Chem.*, **B25**, 327 (1934); Bonino, *Gazz. chim. ital.*, **65**, 5 (1935).

Kohlrausch, Pongratz and Seka⁸, Médard^{9a} and Lu^{9b}; that of thiophene by Venkateswaren¹⁰ and Bonino and co-workers⁷; and that of pyrrole and derivatives by Bonino and co-workers¹¹. In each case the predominant form of the parent nucleus has been reported as one which appears not to have the 1600 line which is usually characteristic of the C = C linkage, although in thiophene a 1408 line was considered as possibly being due to the olefinic linkage. On the basis of the general similarities of these heterocycles it might be expected that their Raman spectra would reveal some concordances. This is the case, as is illustrated in table 1.

Bonino and co-workers have postulated an equilibrium of electronic formulas for these compounds^{7, 11} and believe that the C = C linkage is absent in the parent heterocycle but that the derivatives exist in the ordinary double bonded form. Hausser¹², however, has shown that in certain open chained conjugated systems the conjugated double bonds form a simple system which acts as a whole and that there is a material decrease in Raman frequency as the conjugation increases. Truchet and Chapron¹³ have studied cyclopentadiene and related compounds and have concluded that the nucleus HC—CH is characterized by a frequency of



1500, the ethylene frequency here being reduced by the oscillations of the conjugated double bonds. Thus cyclopentadiene itself has no line in the region of 1600 and its double bonds are decidedly ethylenic and not aromatic in character.

Pauling and Sherman¹⁴, on the basis of thermochemical data, have suggested that furan, thiophene and pyrrole exist in several electronic structures. The differences between the observed energies of formation and those calculated for the various electronic structures have been interpreted as resonance energy of the molecule between two or more structures. Also, on the basis of chemical behavior, dynamic equilibria have been suggested for furan and thiophene types¹⁵.

A study of the parachors of some furans reveals two pertinent points in connection with Raman spectra measurements. First, it appears unlikely that there is contained in furan any large quantity of forms having one or two semipolar bonds. A calculation of the parachor for furan, on the assumption that it has the diolefinic form, gives a value of 160.1. If semipolar bonds are assumed, the calculation gives a value of 120.1 for the parachor of furan.

⁸ Kohlrausch, Pongratz and Seka, *Ber.*, **66**, 11 (1933).

⁹ (a) Médard, *Bull. soc. chim.* [5], **1**, 934 (1934); (b) Lu, *Sci. Rep. Nat. Tsing Hau Univ.*, **1**, 25 (1931) [*C. A.* **26**, 1515 (1932)].

¹⁰ Venkateswaren, *Indian J. Phys.*, **5**, 146 (1930); Venkateswaren and Bhagavan-tam, *ibid.*, **7**, 585 (1933).

¹¹ Bonino, Manzoni-Ansidei and Pratesi, *Z. physik. Chem.*, **B22**, 21 (1933) and **B25**, 348 (1934).

¹² Hausser, *Z. tech. Physik.*, **15**, 10 (1934).

¹³ Truchet and Chapron, *Compt. rend.*, **198**, 1943 (1934).

¹⁴ Pauling and Sherman, *J. Chem. Phys.*, **1**, 606 (1933). See, also, Ingold, *J. Chem. Soc.*, 1120 (1933).

¹⁵ Gilman and Wright, *Chem. Reviews*, **11**, 324 (1932).

A semipolar bond might be expected to depress the parachor, but the observed value of 160.4 is within the experimental error for the diolefinic form⁴. Second, the parachors show no discontinuity or change in nuclear structure in going from furan to its various derivatives as suggested by some investigators on the basis of Raman spectra⁷. The parachor of furan as well as of its derivatives agree rather satisfactorily with the diolefinic form or possibly with a modification of it, such as the centric form.

EXPERIMENTAL PART

The several furans were prepared by standard procedures, and purification was effected by repeated fractional distillation from apparatus free of mineral acid. A trace (about 0.01%) of hydroquinone was helpful in most cases as a stabilizer.

In these orienting experiments the spectra were obtained on Eastman I-J plates by means of a Hilger E1 quartz spectrograph. Densitometer tracings were made from the spectra and measurements of Raman numbers were made from the tracings. The dispersion gave about 85 wave numbers per mm. on the plate and the tracings were made on a ratio of 10 to 1 to give about 8.5 wave numbers per mm. for the region 4300 to 5000. Exposures of 48 hours were made in most cases with the slit of the spectrograph open to about 0.04 mm.

The excitation unit consisted of a sheet aluminum cylindroidal reflector of dimensions to fit a glass cell 30 cm. in length and 1 cm. in diameter placed on one focus line, and a straight tube mercury arc light 30 cm. in length and 2 cm. in diameter along the other focus line. The cell containing the liquid was fitted with a flat window on one end and a water jacket over its entire length. All glass parts were of Pyrex. In operation, the spectrograph was tilted slightly to bring its axis parallel to that of the mercury arc and the window of the cell was placed about one mm. from the slit with the cell along the axis of the spectrograph. The arc light operated on a direct current of 3 amperes.

Figure 1 is a graphical representation of the Raman spectra of the furans studied. The height of the lines represents the comparative intensities as recorded by the densitometer.

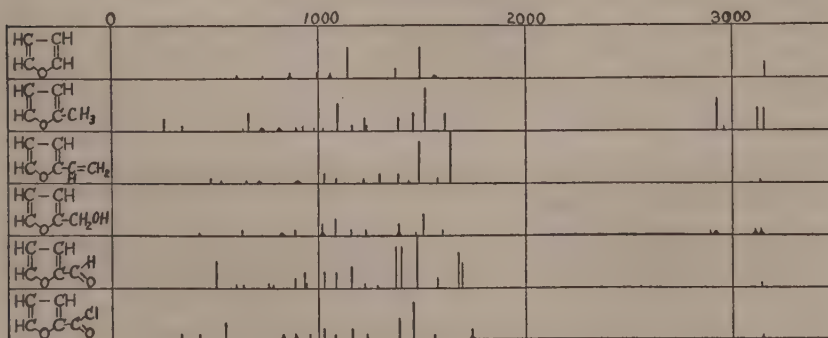


Figure 1—Raman Spectra of Some Furans

TABLE 1. *Comparative Raman lines of furan, thiophene and pyrrole*

| Furan $\Delta \nu^*$ | Thiophene $\Delta \nu^*$ | Pyrrole $\Delta \nu^*$ |
|-------------------------|-----------------------------|---------------------------|
| | 303 (00) | |
| | 356 (00) | |
| | 457 (0) | |
| | 570 (00) | |
| 603 (0) | 606 (10) | 621 (2) |
| | 690 (2) | |
| 730 (0) | 751 (8) | 706 (1½) |
| | | 828 (1) |
| 863 (1b) | 834 (10) | 852 (1) |
| | 875 (1) | |
| | 906 (2) | |
| 996 (1) | 1035 (10) | 1035 (½) |
| 1058 (1b) | 1084 (10) | 1078 (½) |
| 1140 (6) | 1131 (1) | 1140 (8) |
| | 1169 (2) | |
| 1370 (2) | 1361 (10) | 1377 (3½) |
| | 1408 (10) | |
| 1487 (6) | | 1466 (3) |
| 1559 (00b) | | |
| | 2999 (3) | |
| | 3081 (8) | 3073 (1) |
| 3158 (3) | 3104 (10) | 3123 (3) |
| | | 3380 (2b) |

* Raman Frequencies ($\Delta \nu$) in cm^{-1} .

The values in parentheses are intensities; the thiophene numbers are those of Venkateswaren¹⁰, and the pyrrole numbers those of Bonino and co-workers¹¹.

The numbers reported by Glockler and Wiener⁵ for furan are: 604, 735, 857, 990, 1055, 1139, 1381, 1486 and 3165.

TABLE 2. *Raman lines of substituted furans*

2-METHYLFURAN: 256 (2), 345 (1), 635 (0), 661 (3), 726 (0b), 809 (0b), 892 (0), 926 (0), 979 (0), 1020 (0), 1090 (5), 1162 (1), 1221 (2), 1230 (1), 1385 (2), 1456 (3), 1515 (8), 1610 (3), 2928 (6), 2960 (0), 3125 (4), 3154 (4).

2-FURYLETHYLENE: 482 (1), 35 (0), 655 (0), 715 (0b), 900 (0vb), 1028 (2), 1084 (1), 1218 (1), 1295 (2), 1386 (2), 1436 (0), 1484 (8), 1577 (1), 1637 (10), 3143 (0).

2-FURYL CARBINOL: 423 (0), 630 (1), 820 (0b), 885 (1), 1015 (2b), 1081 (3), 1156 (1), 1227 (1), 1383 (2b), 1465 (0), 1502 (4), 1599 (1), 2890 (0), 2921 (0b), 3110 (1b), 3140 (1b).

2-FURFURAL: 503 (5), 599 (0), 635 (0), 756 (1), 780 (0), 885 (2), 931 (3), 940 (1), 1024 (3), 1081 (3), 1156 (4), 1223 (1), 1280 (0), 1370 (8), 1395 (8), 1472 (10), 1569 (2), 1673 (7), 1691 (5), 3142 (1).

2-FUROYL CHLORIDE: 335 (1), 425 (1), 550 (3), 827 (1b), 888 (1b), 957 (1), 1028 (2), 1082 (1), 1160 (2), 1230 (1), 1388 (4), 1456 (7), 1560 (1), 1740 (2b), 3150 (0).

SUMMARY

The Raman spectra of furan and some of its derivatives have been measured, and attention has been directed to some correlations of spectra with related heterocycles (thiophene and pyrrole) which also have enhanced aromatic characteristics.

The foregoing results may be interpreted as in support of the classical diolefinic structure for furan.

ACKNOWLEDGMENT

The authors are grateful to Dr. Henry Gilman and to Dr. William H. Jennings for assistance; and to W. J. Meyer and George Brown for the preparation of two of the compounds, and to the Miner Laboratories and the Quaker Oats Company for the initial furan compounds used in some of the syntheses.

THE DISSIMILATION OF PYRUVIC ACID BY *LACTOBACILLUS LYCOPERSICI*¹

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The true lactic acid bacteria may be classified into two natural groups with respect to their dissimilation of carbohydrates. Orla-Jensen (1919) has placed in different genera those organisms that convert glucose primarily into lactic acid and those which produce, in addition, appreciable quantities of ethyl alcohol, volatile acids and carbon dioxide. Kluyver and Donker (1924) have suggested the terms homofermentative and heterofermentative, respectively, for the two groups.

In view of the intermediary role assigned to pyruvic acid in bacterial metabolism, the mechanism of its breakdown by bacteria is of importance to an understanding of the dissimilation of carbohydrates.

The organism used in this investigation, *Lactobacillus lycopersici*, is typical of the heterofermentative group. It was isolated by Mickle (1924) and described as a gram positive, non-sporeforming, non-motile rod, occurring singly or in chains, and often developing long filaments. Lactic, acetic and carbonic acids, ethyl alcohol and glycerol are formed from glucose. In addition, mannitol is formed from levulose. A detailed description of the organism is given by Pederson (1929).

METHODS

The medium used in this investigation consisted of sodium pyruvate 0.67 per cent, K_2HPO_4 0.6 per cent, and KH_2PO_4 0.6 per cent, yeast extract (Difco) 0.2 per cent and peptone 1.0 per cent. The solutions of (1) yeast extract and peptone and (2) phosphates, were sterilized separately by autoclaving at 20 lbs. for 20 minutes. The sodium pyruvate was sterilized by filtration through a Seitz filter. The ingredients of the medium were combined in sterile 1-liter Erlenmeyer flasks and the pH adjusted to 6.2 at the time of inoculation. Twenty-five cc. from a three-day culture of *L. lycopersici* grown in medium of the same composition were used for inoculation. The fermentations were incubated at 30°C. for 21 days.

Oxygen-free nitrogen was continuously bubbled through the medium to insure strictly anaerobic condition as well as to carry the carbon dioxide produced by the fermentation into Bowen potash bulbs for gravimetric determination.

Upon completion of the fermentation the medium was quantitatively analyzed for lactic, acetic, pyruvic and carbonic acids. The lactic acid was determined by the Friedemann, Cotonio and Shaffer (1927) method. The volatile acids were determined by steam distilling 400 cc. of the fermented liquor to two liters of distillate. Owing to the slight volatility of

¹Supported in part by a grant from the Industrial Science Research fund and in part by the Rockefeller Fluid Research fund of Iowa State College.

the pyruvic acid it was necessary to neutralize the distillate with sodium hydroxide, evaporate to 300 cc., acidify and again steam distill to recover the volatile acids. The acids in the second distillate were determined by the partition method of Osburn, Wood and Werkman (1933). The pyruvic acid was continuously extracted with ethyl ether from an aliquot part of the medium and determined by iodoform titration, according to Wendel (1931). The residual carbon dioxide in the medium was driven from the liquor by acidifying to congo red with sulfuric acid and boiling under a reflux condenser. The carbon dioxide was carried by CO₂-free air into Bowen potash bulbs and determined gravimetrically.

Purity of the cultures was assured by microscopic and cultural examination at the time of inoculation and just prior to analysis.

EXPERIMENTAL

The results of two representative experiments are presented in table 1. The data in the first column are expressed in millimols per liter of medium; in the second column in millimols of carbon. The oxidation-reduction ratios are given for each experiment.

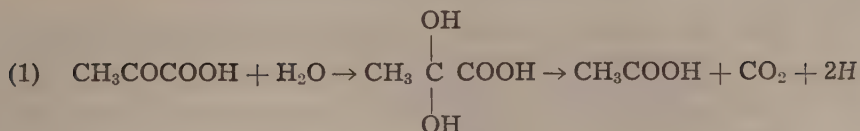
No glycerol, ethyl alcohol or succinic acid was detected in the fermentations. The creatine test for acetoin was negative.

TABLE 1. Dissimilation of pyruvic acid by *Lactobacillus lycopersici*

| | I | | II | |
|----------------------------|-------------|-----------------|-------------|-----------------|
| | mM liter | mM of carbon | mM liter | mM of carbon |
| Pyruvic acid fermented | 70.5 | 211.5 | 46.7 | 140.1 |
| Lactic acid | 37.7 | 113.1 | 22.8 | 68.4 |
| Acetic acid | 35.1 | 70.2 | 22.6 | 45.2 |
| Carbon dioxide | 38.8 | 38.8 | 23.8 | 23.8 |
| Total | | 222.1 | | 137.4 |
| Percentage of C. recovered | | 105.2 | | 98.1 |
| Oxidation-reduction index | | 1.10 | | 1.02 |

The three compounds resulting from the fermentation were found to occur in equimolar quantities, suggesting a dissimilation involving the oxidation of one molecule with the simultaneous reduction of another molecule of pyruvic acid.

Decarboxylation of the pyruvic acid does not appear to occur since fixation of acetaldehyde by dimedon or bisulfite was unsuccessful after repeated trials. The close relationship of the lactic to the propionic acid bacteria and the failure of van Niel (1928) and Wood and Werkman (1934) to detect acetaldehyde in fermentations by the latter organisms suggests the applicability of the proposal of these investigators to the lactic acid bacteria; that is, a hydration of the pyruvic acid occurs followed by a dehydrogenation resulting in the formation of carbon dioxide and acetic acid as shown in equation (1). The active hydrogen formed serves to reduce a second molecule of pyruvic acid to lactic acid (equation 2).



Simon (1932) assigned an intermediary role to pyruvic acid in the fermentation of lactic acid by *L. delbrückii*, a homofermentative lactic acid organism. Nelson and Werkman (1935) have fixed pyruvic acid with calcium sulfite in the fermentation of lactic acid by members of the heterofermentative group. They suggested that the active hydrogen formed from the breakdown of the lactic acid and also from the breakdown of intermediary pyruvic acid serves in part to reduce glyceric aldehyde to glycerol. On the other hand, the data in table 1 show that a second molecule of pyruvic acid accepts the hydrogen formed from the breakdown of a first molecule of the compound. In view of the presence of hydrogen acceptors in addition to pyruvic acid, in the fermentation of glucose, it is probable that pyruvic acid and glyceric aldehyde as well as acetaldehyde play the role of hydrogen acceptors.

It is interesting to note the similarity of the fermentation of levulose and of pyruvic acid by the heterofermentative bacteria. Each compound acts as a hydrogen donor as well as an acceptor. Levulose is dissimilated to lactic acid, acetic acid, carbon dioxide and ethyl alcohol as well as reduced to mannitol. Pyruvic acid forms acetic acid and carbon dioxide and is reduced to lactic acid. This ability with respect to levulose seems to be quite specific for the heterofermentative lactic acid bacteria. Whether the ability of the organisms to bring about the simultaneous oxidation and reduction of pyruvic acid is specific for this group of lactic acid organisms has not been determined.

CONCLUSIONS

Pyruvic acid was fermented by *L. lycopersici* with the production of lactic, acetic and carbonic acids in equimolar quantities.

One molecule of pyruvic acid undergoes oxidation to acetic and carbonic acids with the simultaneous reduction of a second molecule of pyruvic to lactic acid.

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THE ACID AND ALKALINE DEGRADATION OF CHLORINATED WOOL¹

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Chlorination of wool, suggested about 1839 by Mercer (14) as a means of increasing its affinity for dyes and introduced into practice in 1865 by Lightfoot (12), has also been used in producing an unshrinkable finish. Although chlorinated wool varies with chlorinating agent or degree of chlorination, it has been compared with wool as of greater affinity for water and dyes, less tendency to shrink and felt, greater luster and scroop, less tensile strength and elasticity, and greater solubility in water and dilute acid and alkali.

Trotman (18) has reported gradual solution of nitrogen and sulfur from wool and change in the ratio of sulfur to nitrogen in the residual wool with increasing time or temperature of chlorination; he has noted solution of 2.6 and 4.8 per cent of wool in 0.1125 *N* and 0.1687 *N* hypochlorous acid and 12.0 per cent of sulfur and 2.7 per cent of nitrogen in 0.2812 *N* hypochlorous acid. Sobue and Hirano (16) have reported solution of 2.25 and 5.47 per cent of wool in one hour at 25°C. in calcium hypochlorite corresponding to 0.0032 *N* and 0.1428 *N* hypochlorous acid. Wool has been described as losing no nitrogen when treated thirty minutes with hypochlorous acid, 0.5 per cent chlorine per weight of wool (20), and 1.4 per cent nitrogen upon 10 per cent chlorination (5). Barritt and King (6) have decreased the sulfur 28.1 per cent in one hour with 31.2 per cent of chlorination per weight of wool, although Haller (11) has considered sulfur not connected with the reducing properties of wool.

Trotman (18) has described chlorinated wool as no more and over-chlorinated wool as much more soluble than wool in water, dilute acetic acid, 0.1 *N* sodium carbonate or sodium hydroxide, measured by loss in weight or colorimetric estimation of dissolved nitrogen, but as more soluble than wool in 0.5 *N* sodium carbonate in twelve hours (19).

To obtain quantitative data of the effect of dilute acid and alkali on the composition and mechanical failure of chlorinated wool at temperatures of use and dyeing we have chlorinated wool with 0.06 *N* hypochlorous acid at 25°C. for an hour, treated it with 0.5 to 6 *N* hydrochloric acid for ten hours at 25°C., with 0.25 to 0.75 *N* hydrochloric acid for one hour at 100°C., and with 0.05 *N* to 0.2 *N* sodium hydroxide for ten hours at 40°C., and determined the weight, nitrogen, sulfur, and wet strength of the residual wool.

EXPERIMENTAL PROCEDURE

Preparation of Wool

Plain-woven wool was boiled one hour in one hundred volumes of water, rinsed, dried at room temperature, cut for analysis, extracted con-

¹ Journal Paper No. J281 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 381.

tinuously with anhydrous ether for eighteen hours and, excepting the specimens for breaking strength, dried at 105° to 110°C. until successive weighings with tare agreed within half a milligram.

Preparation of hypochlorous acid

A solution of 65 grams of sodium carbonate was added to 100 grams of bleaching powder in warm water, diluted to two liters, and filtered. To a measured volume of the filtrate an equivalent amount of dissolved boric acid was added just before use. The iodine freed when hypochlorous acid was added from a burette to 25 cc. of ten per cent potassium iodide, acidified with hydrochloric acid and diluted to half a liter, was titrated with standard sodium thiosulfate using starch paste as an indicator (17). The concentration of the hypochlorous acid was checked electrometrically by Penot's method (7).

Chlorination of wool

Five-gram samples of wool were immersed in 200 cc. of water or standard hypochlorous acid in a stoppered flask at $25^{\circ} \pm 0.1^{\circ}\text{C.}$ for one hour and washed in water until the rinse no longer produced an opalescence with silver nitrate. The effect of hypochlorous acid on a wool used for preliminary tests is shown in table 1. The change in wool brought about by 0.06 *N* hypochlorous acid in one hour at 25°C. is shown in table 2.

TABLE 1. *Effect of hypochlorous acid on the weight, nitrogen, sulfur, and breaking strength of wool in one hour at 25°C.*

| Hypchlorous acid | Residual wool | | | |
|------------------|--------------------|----------|--------|-------------------------------|
| | Weight | Nitrogen | Sulfur | Breaking strength of wet warp |
| normality | percentage of wool | | | pounds per inch |
| 0 | 99.7 | 16.27 | 3.62 | 13 |
| 0.0133 | 99.4 | 16.26 | 3.62 | 12 |
| 0.0528 | 97.9 | 16.26 | 3.49 | 12 |
| 0.0806 | | | | 10 |
| 0.1209 | | | | 9 |
| 0.1377 | | | | 8 |

Acid degradation of wool

Five grams of wool were immersed in 200 cc. of water or hydrochloric acid, standardized by precipitation as silver chloride, in a stoppered flask at $25^{\circ} \pm 0.1^{\circ}\text{C.}$ for ten hours and washed in water until the rinse gave no test for chloride. Tests at 100°C. for one hour were made with water or hydrochloric acid in balloon flasks fitted with water-cooled reflux condensers and heated in a boiling water bath (3).

TABLE 2. *Change in wool brought about by 0.06 N hypochlorous acid in one hour at 25°C.*

| Determination | Wool | Chlorinated wool |
|--|-------|------------------|
| Ash, percentage | 0.07 | none |
| Nitrogen, percentage | 16.31 | 15.98 |
| Breaking strength of wet warp, pounds per inch | 12 | 10 |
| Sulfate sulfur (12), percentage | 0.36 | none |
| Sulfite-yielding sulfur (9), percentage | none | none |
| Total sulfur, percentage | 4.07 | 3.72 |
| Weight, percentage | 100.0 | 98.4 |

Alkaline degradation of wool

Five grams of wool were immersed in 200 cc. of water or standard sodium hydroxide in a stoppered flask at $40^{\circ} \pm 0.1^{\circ}\text{C.}$ for ten hours and washed in water until the rinse gave no test for alkali with phenolphthalein.

Analysis of wool

The wet warp breaking strength of ten specimens was determined at once with a Scott Universal Tester (1). The wool was dried at room temperature (15) before analysis for nitrogen by the Kjeldahl-Gunning method (9) and at 105° to 110°C. before analysis for sulfur by the Benedict-Denis method (8, 4). Each value for weight, nitrogen, sulfur or ash is the average of four determinations.

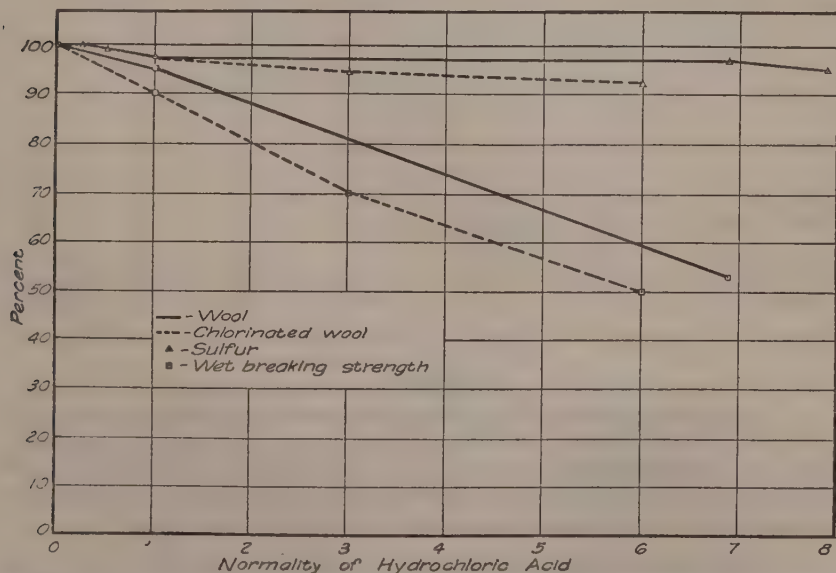
Fig. 1. Effect of hydrochloric acid in ten hours at 25°C. on the sulfur and wet breaking strength of wool and chlorinated wool.

Table 3 shows the effect of acid and alkaline degradation on the weight, nitrogen, sulfur, and wet strength of the chlorinated wool. Hydrochloric acid in ten hours at 25°C. affected chlorinated wool the same as wool, measured by weight and nitrogen (3); its greater effect on the sulfur and wet strength of the chlorinated wool is shown in fig. 1. Chlorinated wool was more degraded than wool by hydrochloric acid in one hour at 25°C., especially as measured by loss of sulfur (fig. 2). In ten hours at 40°C. sodium hydroxide decreased the nitrogen and weight of chlorinated wool to the same extent and much more than in the case of wool (fig. 3) although it decreased the sulfur of both wools to the same extent (2). The wet strength of chlorinated wool was completely destroyed in one hour at 40°C. by 0.05 *N* sodium hydroxide.

TABLE 3. *Effect of acid and alkali on the weight, nitrogen, sulfur, and breaking strength of chlorinated wool*

| Hydrolytic agent | Time | Temperature | Residual wool | | | |
|-------------------|------|-------------|--------------------------------|----------|--------|-------------------------------|
| | | | Weight | Nitrogen | Sulfur | Breaking strength of wet warp |
| Normality | hour | °C. | percentage of chlorinated wool | | | pounds per inch |
| Hydrochloric acid | 10 | 25 | 97.1 | 15.84 | 3.69 | 10 |
| | | | 97.1 | 15.75 | 3.54 | 10 |
| | | | 96.8 | 15.80 | 3.60 | 9 |
| | | | 95.8 | 15.49 | 3.49 | 7 |
| | | | 91.4 | 14.77 | 3.42 | 5 |
| | 1 | 100 | 94.6 | 15.72 | 3.68 | 10 |
| | | | 89.8 | 14.67 | 3.34 | 5 |
| | | | 84.5 | 13.42 | 3.15 | 1 |
| | | | 78.5 | 12.30 | 3.06 | <1 |
| | | | | | | |
| Sodium hydroxide | 10 | 40 | 96.4 | 15.71 | 3.67 | 10 |
| | | | 89.4 | 14.79 | 2.88 | <1 |
| | | | 84.7 | 13.76 | 2.29 | <1 |
| | | | 75.5 | 12.60 | 1.88 | <1 |
| | | | 66.0 | 10.82 | 1.67 | <1 |
| | | | | | | |

SUMMARY

1. Degradation of wool, chlorinated by 0.06 *N* hypochlorous acid in one hour at 25°C., by 0.5 to 6 *N* hydrochloric acid in ten hours at 25°C., 0.25 to 0.75 *N* hydrochloric acid in one hour at 100°C., and 0.05 to 0.2 *N* sodium hydroxide in ten hours at 40°C. has been followed by the weight, nitrogen, sulfur and wet breaking strength of the residual wool.

2. Degradation of chlorinated wool by acid in ten hours at 25°C. exceeded that of wool as measured by sulfur or wet strength; at 100°C. the loss in sulfur was greater than that in weight, nitrogen or wet strength.

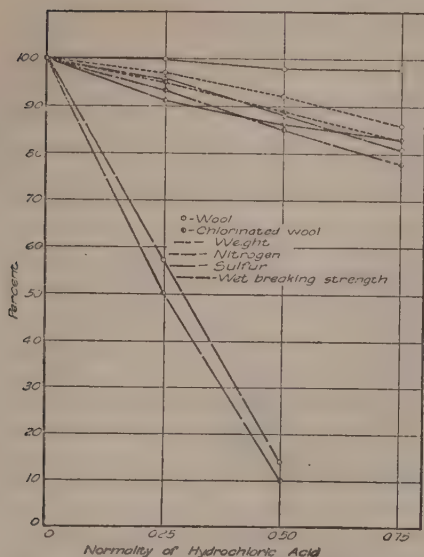


Fig. 2. Effect of hydrochloric acid in one hour at 100°C. on the weight, nitrogen, sulfur, and wet breaking strength of wool and chlorinated wool.

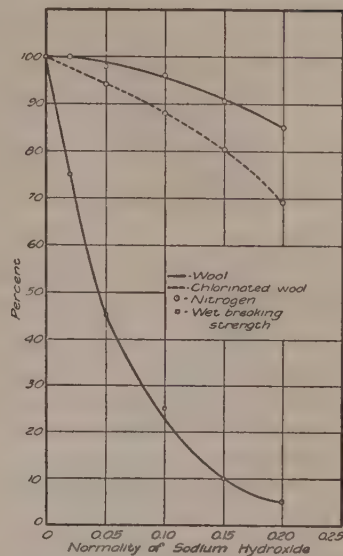


Fig. 3. Effect of sodium hydroxide in ten hours at 40°C. on the nitrogen and wet breaking strength of wool and chlorinated wool.

3. Sodium hydroxide in ten hours at 40°C. dissolved the same amount of sulfur from chlorinated wool as from wool but decreased the nitrogen and weight of the chlorinated wool more. The wet strength of chlorinated wool was completely destroyed in one hour at 40°C. by 0.05 N sodium hydroxide.

4. The residual chlorinated wool has been shown to decrease in nitrogen and sulfur with increasing concentration of acid or alkali.

ACKNOWLEDGMENT

We wish to thank Miss Eleanor Winton for the sulfur determinations and Mrs. Paul Van Ess for the nitrogen determinations reported in table 1.

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FUNGI ASSOCIATED WITH TREE CANKERS IN IOWA I. PRELIMINARY SURVEY¹

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The establishment of Civilian Conservation Corps camps as a part of the Emergency Conservation Work program of the Federal government in Iowa afforded an opportunity to study the decline of trees in Iowa parks and shelter belts. Technical foremen trained in the field of plant pathology were placed in 17 camps throughout the state to conduct surveys for plant diseases including white pine blister rust and the Dutch elm disease and to eradicate the more important diseases wherever sanitation was feasible. In conducting the survey these men soon found that field identification of the numerous pathological specimens was impossible and arrangements were therefore made to have this material forwarded to the Botany Department at Ames for identification.

The men were instructed to collect material only from standing trees in the early stages of decline and to send in with the specimens a form showing host identity, locality and time of collection. Their identification of the host, description of the disease and estimate of severity were accepted. Examination of the specimens soon disclosed a preponderance of twig and branch injury chiefly in the form of lateral or encircling cankers which were fatal to the terminal section.

Various fungi were found to be fruiting either in the dead or partially killed tissues surrounding the killed area of the canker. The identification of these fungi was undertaken as a preliminary step to the study of the pathological condition. Fungi were identified on 713 of the 860 specimens which were received before the suspension of the field foremen abruptly ended the program.

This paper is a summary of these 713 identifications. The collections upon which these identifications are based are preserved in the herbarium at Iowa State College. The report is to be considered only as a preliminary statement, since, as can be judged from the number of specimens examined and the limited time which has elapsed since the beginning of the program, much checking of synonymy, and more intensive examination of the material still remains to be done. In spite of these shortcomings it was deemed desirable to make a progress report at this time. As soon as each group of fungi has been checked thoroughly, it and the allied species previously described from Iowa will be presented with adequate descriptions.

The 860 specimens represented 20 families, 36 genera and 68 species. As might be expected from the emphasis that has been placed on white pine blister rust and Dutch elm disease, *Pinus strobus* L. and *Ulmus*

¹ Journal Paper No. J289 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 362.

americana L. were most abundantly represented with 104 and 100 specimens, respectively. However, 25 different fungi were found present on the American elm, while but 12 species were identified from the pine. The ten host species most frequently encountered are listed in table 1.

TABLE 1. The ten most commonly submitted hosts and the genera of the fungi containing the largest number of species found on 713 specimens

| Hosts | Specimens examined | Infected | Genera of fungi | Number of species |
|----------------------------|--------------------|----------|-----------------------|-------------------|
| <i>Pinus strobus</i> | 104 | 78 | <i>Cytospora</i> | 25 |
| <i>Ulmus americana</i> | 100 | 51 | <i>Sphaeropsis</i> | 16 |
| <i>Populus deltoides</i> | 59 | 48 | <i>Tubercularia</i> | 16 |
| <i>Acer saccharinum</i> | 55 | 42 | <i>Diaporthe</i> | 12 |
| <i>Fraxinus lanceolata</i> | 43 | 28 | <i>Dothiorella</i> | 10 |
| <i>Populus tremuloides</i> | 32 | 29 | <i>Valsa</i> | 8 |
| <i>Pinus sylvestris</i> | 29 | 22 | <i>Coniothyrium</i> | 5 |
| <i>Fraxinus americana</i> | 24 | 20 | <i>Didymosphaeria</i> | 5 |
| <i>Salix nigra</i> | 21 | 18 | <i>Diplodia</i> | 5 |
| <i>Acer negundo</i> | 20 | 13 | <i>Melanconium</i> | 5 |

The range of fungi was even greater than that of the hosts. The 213 identifiable species were distributed in 87 different genera. These were distributed among 15 families of Ascomycetes, 5 families of the Fungi imperfecti and one family of Basidiomycetes; the majority were to be found in the orders Sphaeriales and Sphaeropsidales. The genus most frequently met was *Cytospora* with 25 species on 47 hosts. *Sphaeropsis* and *Tubercularia* with 16 species on 27 and 28 hosts, respectively, were second in frequency. The ten genera with the largest number of different species are listed in table 1.

If we change the point of view and examine the combinations of host and fungus most frequently collected (table 2) we find that *Scolecnectria scolecospora* (Bref.) Seaver on *Pinus strobus* heads the list, having been collected 21 times. *Valsa pini* (A. & S.) ex Fr. on the same host is second with 18 collections and *Cytospora pinicola* West., which is considered by many mycologists as the conidial stage of the *Valsa*, follows with 16 specimens. Combined, these two fungi were present in 34 collections and should perhaps be first. *Cytospora annularis* Ell. & Ev. on green ash, *Fraxinus pennsylvanica* Marsh var. *lanceolata* (Borkh.) Sarg., followed, being found 11 times. *Sphaeropsis ellisii* Sacc. appeared 10 times on both *Pinus strobus* and *Pinus sylvestris* Linn. *Cytospora nivea* (Hoffm.) Sacc. was present 9 times on *Populus alba* L., *Populus deltoides* Marsh, and *Populus tremuloides* Michx. *Sphaeropsis inquinans* Pk. was identified a like number of times (9) on *Acer saccharinum* L. Although *Ulmus americana* was received in great numbers, the number of different parasites on it was so great that the first occurrence of this host on this list was in sixteenth place, where it appears as a host for *Cytospora carbonacea* Fr. in six instances.

An analysis of these figures indicates that white pine is among the trees most seriously threatened by disease. This species is closely followed by green ash and the poplars. *Acer saccharinum* is another species which

seems to be highly susceptible. Scotch pine is somewhat more resistant. The American elm is quite resistant to disease. At least the fact that there are a large number of fungi, none of which have gained a dominance, would indicate such a conclusion.

TABLE 2. *Combinations of fungus and host most frequently collected*

| Fungus | Host | Number of collections |
|-------------------------------------|--|-----------------------|
| <i>Scolecconectria scolecospora</i> | <i>Pinus strobus</i> | 21 |
| <i>Valsa pini</i> | <i>Pinus strobus</i> | 18 |
| <i>Cytospora pinicola</i> | <i>Pinus strobus</i> | 16 |
| <i>Cytospora annularis</i> | <i>Fraxinus pennsylvanica</i> var. <i>lanceolata</i> | 11 |
| <i>Sphaeropsis ellisii</i> | <i>Pinus strobus</i> | 10 |
| <i>Sphaeropsis ellisii</i> | <i>Pinus sylvestris</i> | 10 |
| <i>Cytospora nivea</i> | <i>Populus alba</i> | 9 |
| <i>Cytospora nivea</i> | <i>Populus deltoides</i> | 9 |
| <i>Cytospora nivea</i> | <i>Populus tremuloides</i> | 9 |
| <i>Sphaeropsis inquinans</i> | <i>Acer saccharinum</i> | 9 |
| <i>Valsa pini</i> | <i>Pinus sylvestris</i> | 8 |
| <i>Cytospora salicis</i> | <i>Salix nigra</i> | 8 |
| <i>Dothichiza populea</i> | <i>Populus deltoides</i> | 7 |
| <i>Cytospora chrysosperma</i> | <i>Populus deltoides</i> | 7 |
| <i>Cytospora annularis</i> | <i>Fraxinus americana</i> | 6 |
| <i>Cytospora carbonacea</i> | <i>Ulmus americana</i> | 6 |
| <i>Steganosporium cellulosum</i> | <i>Acer saccharum</i> var. <i>nigra</i> | 6 |
| <i>Tubercularia vulgaris</i> | <i>Morus rubra</i> | 5 |
| <i>Tubercularia nigricans</i> | <i>Ulmus americana</i> | 5 |
| <i>Valsa nivea</i> | <i>Populus tremuloides</i> | 5 |
| <i>Sphaeropsis simillina</i> | <i>Acer saccharinum</i> | 5 |
| <i>Hypoxylon pruinaum</i> | <i>Populus tremuloides</i> | 5 |
| <i>Didymosphaeria accedens</i> | <i>Fraxinus pennsylvanica</i> var. <i>lanceolata</i> | 5 |
| <i>Cryptosporium coronatum</i> | <i>Populus deltoides</i> | 5 |

As yet we have very little data concerning the parasitism of the fungi involved. Our opinion is that they are chiefly secondary invaders after other injuries. The very fact that of the total number of collections (860) there were 147 which showed no identifiable fungi would indicate that cankers on trees may be caused by other agencies than parasitic fungi. Insects are responsible in some cases. Sunscald is common on smooth barked trees where they are not shaded. Mechanical injuries also occurred. The trees studied were in plantings rather than natural habitats. The pines were not well adapted to the conditions under which they were growing. The poplars and soft maple were reaching their maturities and therefore were in the right condition for the attack by these fungi.

OBSERVATIONS ON THE ONION THIRPS (THRIPS TABACI LIND.)¹

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The present paper deals largely with experiments with onion thrips carried on in conjunction with a survey of the insect pests of the onion. The purpose of the survey was to find the species of insects which are the active agents responsible for the dissemination of the virus of a disease known as yellow dwarf of the cultivated onion. Most of the life history experiments were conducted in the insectary and greenhouse at Ames, Iowa, during the summer of 1928. The field observations were made both at Pleasant Valley and Ames, Iowa.

At the time the yellow dwarf malady was discovered near Pleasant Valley it seemed quite patent that insects were responsible for the natural spread of the disease in the field. When a study of the situation was first started considerable attention was given to the onion thrips and other primary and secondary pests of this plant as possible vectors of the disease. Many tests, however, failed to prove that the adults or nymphs can serve as conveyors of the virus (Drake, Tate and Harris, 1932). In these experiments hundreds of adults, which had been reared solely on diseased onions for one, two or more generations, failed to act as carriers of the disease when transferred to healthy onions. The offsprings of thrips which had fed solely on diseased onions for several generations also failed to transmit the inoculum. In Hawaii the onion thrips (Lindford, 1932) has been convicted of serving as the transmitter of a virus disease of the pineapple, called pineapple yellow spot.

The onion thrips is one of the pests most seriously affecting the onion crop in Iowa. It feeds on all aerial portions of the growing plant and to some extent on the bulbs in storage. Shipments of sets, mother bulbs and commercial onions almost invariably harbor one or more stages of the insect. Numerous outbreaks of the insect have been recorded in the principal onion-growing districts of the state since 1894. On account of a very heavy infestation at the time yellow dwarf was discovered at Pleasant Valley, the onion thrips was generally suspected and often wrongly accused of being responsible for the natural spread of the malady.

POPULATION COUNTS (Fig. 1)

Many workers on the economy of thrips have considered in some way or other the influence of meteorological factors on the population-building of these insects. In the case of *Thrips tabaci* on the onion the recurrence of epidemics has not infrequently been correlated with subnormal rain-

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fall and relatively high temperature during the vegetative period of this plant. In order to secure more definite information regarding the rate of population increase and the effects of temperature and rains upon actual infestations, ten onion plants were selected each day at random in the field and then the adults and larvae feeding upon the exposed foliage of these plants were removed and counted. As soon as the thrips from the exposed surfaces of a given onion were collected, the plant was pulled and immediately torn apart over a suitable surface so that individuals which had secreted themselves in the axils of the leaves or other parts were included in the counts. Records for each onion plant were kept separately. As the plants were destroyed in making the counts, it was necessary to select a new series of 10 plants each day. Yellow bottleneck onions, grown from sets in the experimental field at Ames, were used for making the studies. The counts, started on June 12 and continued until two days

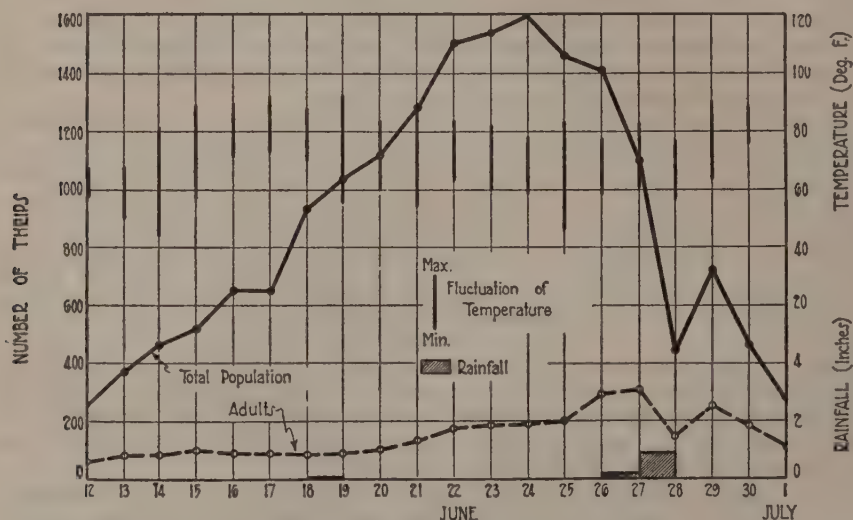


Fig. 1. Graph depicting seasonal trend in plant infestation and effect of driving rains thereon.

before the crop was harvested on July 4, were taken at daily intervals. In the accompanying graph (fig. 1) the trend of population from day to day as evidenced by the counts is depicted. The rate of increase was very rapid and reached its peak on June 24, when 1,596 thrips were collected from 10 plants. The sharp numerical decline of the population on June 26 and 27 was the result of driving rains, which on the latter date had been accompanied by a heavy hail storm. The sudden rise on the following day is accounted for partly by the emergence of new adults, which as the curve shows had been rather high up to this time, and partly by the re-appearance on the plants of thrips that had been knocked off by the rain yet had survived it.

The effect of hard rains on thrips populations was considerably influenced by the type of soil. On the other hand thin rains (fig. 1, June 18)

had little, if any, measurable effect on the population. During heavy downpours many individuals were beaten to the ground and destroyed and, in soils which had a tendency to pack and cake after such rains, emergence of newly transformed adults and individuals which had sought refuge in crevices in the soil was considerably checked. A grand total of 17,788 thrips—14,832 nymphs and 2,956 adults—was collected in the study. Based on these data the population at the peak of the infestation ran from 15,000,000 to 30,000,000 individuals or more per acre. Under such infestations the effect of the feeding activities of the insect becomes quite pronounced. Onions with thick neck, scullions and small unmarketable bulbs are very common. Leaves of individual plants become speckled, then whitish in color, crinkle, curl, and soon become brown and die.

As is evidenced by the daily records, the season of 1928 was quite ideal for the increase of onion thrips. In contrast the past season, 1934, due to excessively high temperatures and the severe and prolonged drouth, was extremely unfavorable for thrips and as a result the infesta-

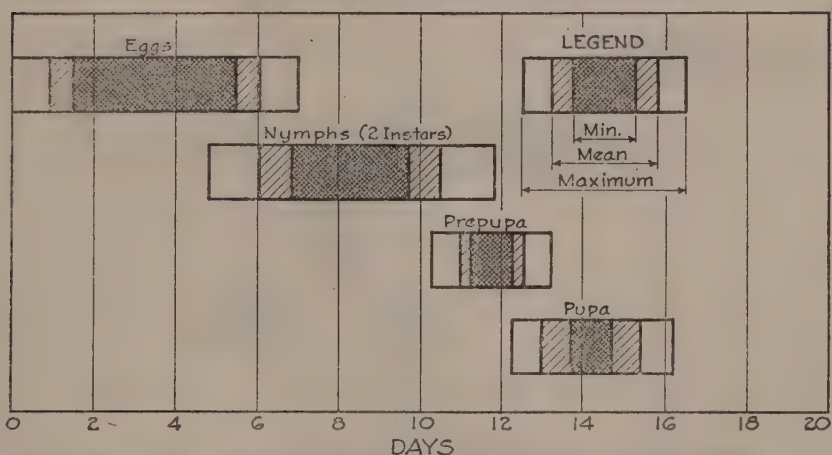


Fig. 2. Duration of life stages of *Thrips tabaci* in an outdoor insectary.

tion was not sufficiently high to allow practical control experiments to be carried on in the field.

INJURY CAUSED BY FEEDING

The feeding activities of individual thrips result in the production of minute lesions on the leaves which develop into necrotic spots. As the population increases, these lesions become more numerous and often confluent, thus producing the well-known condition commonly designated by such appellations as "white blast" and "silver top" (Pl. III, fig. 5). The seriousness of this injury is later manifested by a curling and gradual drying of the foliage and under very severe infestations the premature death of the plant. Both adults and nymphs attack the inflorescence. The importance of this injury in producing onion sterility was treated by Pearson in 1930.

In order to obtain a clearer idea of the damage to the cells and tissues microscopical studies of the lesions were made. Materials were obtained

from plants showing various degrees of feeding injury and, for comparative purposes, from uninfested plants. Small sections of the leaves were killed and fixed in chromacetic solution and stained in iron hematoxylin. Paraffin sections varying from 10-20 microns in thickness were employed in the study, the thicker sections being more desirable for injury examination. Both longitudinal and transverse sections of infested and uninfested tissues are shown in Plate III.

The histology of injured leaves is shown in Plate III, figures 2 and 4. Figure 5 of the same plate shows an enlarged surface view of the feeding injury. It is quite evident that the outer epidermal layer is completely disrupted and the palisade cells badly disorganized and often even entirely destroyed. Occasionally injury extends into the mesophyll cells. The gashing of the epidermis and the piercing and driving of the stylets into the underlying cells result in rapid desiccation of the lacerated tissues and the formation of necrosed areas.

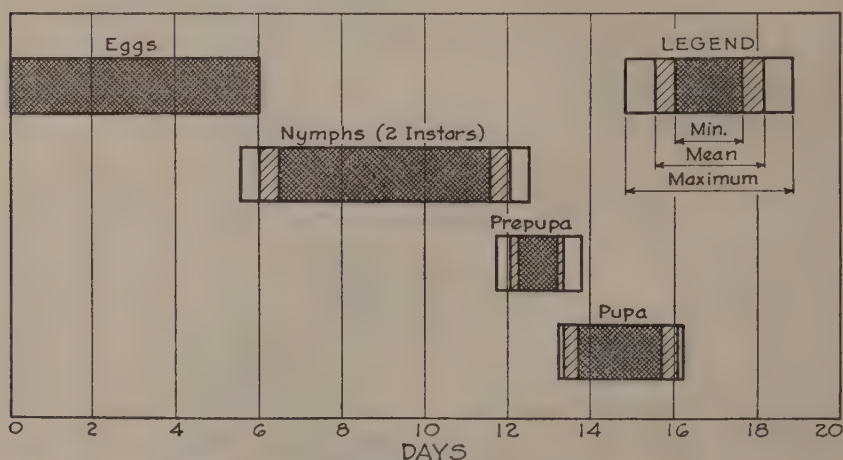


Fig. 3. Duration of life stages of *Thrips tabaci* at 25° C.

SEX RATIO AND PARTHENOGENESIS

From the proportion of males to females collected in field studies it has long been evident that parthenogenesis occurs in the Thysanoptera. Shull (1914) gave a good review of sex in the life cycle of a number of species of thrips. He also made a series of field collections of numerous species in order to determine their sex ratio. Out of 228 specimens of *Thrips tabaci* taken at Douglas Lake and Ann Arbor, Michigan, only two males were recorded.

In connection with the writers' field-infestation counts on onions, a series of 500 adults were found to represent 452 females and 48 males (identification checked by Mr. Dudley Moulton). The question, would the sex ratio be different if the collections were made late in the fall, has not infrequently been raised. With this thought in mind 240 adults were collected on volunteer onions growing at Ames on November 2, 1931. Of this number only 4 were males, representing a ratio of 1 male for 60 imagoes. Shull, in the late autumn, found an increase in the proportion of males to females.

Ten virgin females, which had been confined in separate cages from the time of hatching, were kept on onion leaves known to have always been free from thrips. Four days after reaching the imaginal stage, some of the females began to lay eggs. From the offspring of these females 100 newly hatched nymphs were picked out indiscriminately and then reared to maturity in individual cages². Of these, all were winged males. These results agree with those obtained by Shull (1917) with *Anthothrips verbasci*, by Eddy and Livingston (1931) with *Franklinella fusca*, and by Smith and Nelson (1933) with *Taeniothrips gladioli*. On the other hand Eddy and Clarke (1930) reared *Thrips tabaci* parthenogentially on cotton seedlings and all offspring were females. Similarly Sakimura, in Hawaii, reared 85 progenies from unmated females and all were females (1932). This same author examined 5,000 specimens from various sources, and these were all females save five. In Sudan, South Africa, McGill (1927) collected from cotton, at various times throughout the summer, 3,000

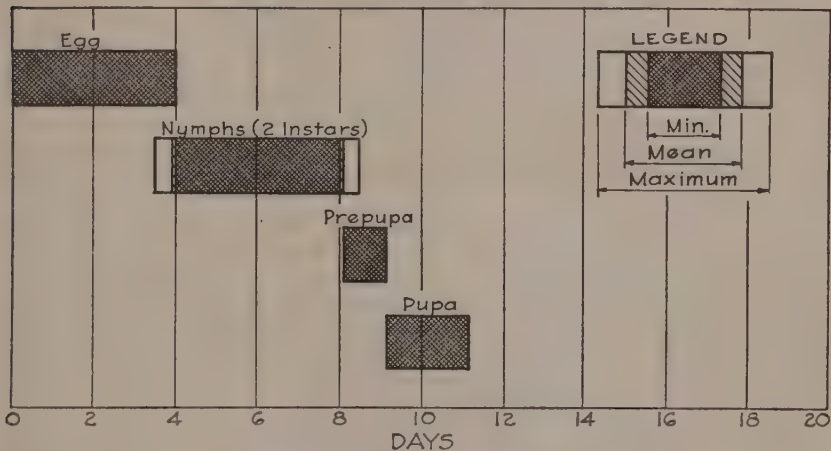


Fig. 4. Duration of life stages of *Thrips tabaci* at 30° C.

individuals without finding a male. Recently one of the writers (H. D. T.) reared a dozen or so offspring from unmated females, all of which were females.

It is thus evident that the sex ratio and method of reproduction, with a consideration of any possible effects of kind and condition of food, and of relative humidity thereon, need further critical study.

LONGEVITY OF THE FEMALE

In order to determine the longevity of the female thrips, 50 newly emerged adults were placed in individual cages and kept in a constant temperature of 30° C. Fresh onion leaves were supplied at regular intervals for food. The maximum length of life was 39 days, the minimum 9 days and the mean 19.9 days. Also, 10 newly emerged adults were placed in separate cages without food and kept at a constant temperature of 30°

² The authors are indebted to Dr. T. A. Brindley, who, while located at Ames, assisted with these studies and made observations.

C. and constant relative humidity of 70 per cent. These lived for an average period of only two days.

LIFE STAGES (Plates I and II)

The onion thrips is world-wide in distribution. It is very common in the United States and often occurs in menacing numbers in onion-growing districts. Onion, cabbage, turnip, beet, tomato, sweet clover and many other cultivated crops, many weeds and numerous other plants serve as suitable hosts.

In these studies the two feeding larval stages are designated as first and second instar nymphs and the two non-feeding larval stages as prepupa and pupa, respectively. The adult female is depicted in Plate I and the egg and four larval stages in Plate II. The eggs are deposited singly in the tissues of the plant. The different stages have been described in detail by Federov and others.

Observations on the life history of the onion thrips in Russia have been published by Lindeman (1888), in Florida by Quaintance (1898), in Massachusetts by Hinds (1902), in Iowa by Horsfall and Fenton (1922) and in South Carolina by Eddy and Clark (1930). A very complete record of its ethology on tobacco in Crimea is given in the excellent paper by Federov (1930). As stated above, the biological observations by the writers were made while attempting to ascertain whether the onion thrips is capable of serving as mechanical or biological carrier of a virus disease of the onion.

For conducting the studies of the activity and life stages of the onion thrips, single individuals were kept in cages made of shell vials 60 mm. in length and 20 mm. in diameter. Small pieces of cellucotton were tamped in the bottom of each vial and then covered with a tightly-fitting disc of blotting paper. The cellucotton and blotting paper were slightly moistened and then at regular intervals remoistened so as to keep the relative humidity comparatively high in the cage. The vials were tightly closed by means of plugs of cotton batting.

Sections of fresh onion leaves approximately 40 mm. in length were used as food. In order to facilitate the observance of the caged insect and at the same time to check the rate of evaporation, the ends of the sections of onion leaves were sealed with paraffin. With this technique it was possible to make observations at any time without removing the insect or the portion of the onion leaf from the vial. Such leaf-sections served as a source of food for a period of 3 to 5 days when kept in the constant temperature and constant humidity cabinets.

From 4 to 6 days after emergence oviposition begins. Eggs of a known age were obtained by confining a number of females for a period on a small section of a fresh onion leaf known to be free from thrips. At the end of 24 hours the thrips were removed from each vial and the sections kept in their respective cages at the desired temperatures until the end of the incubation period.

The egg at the time of laying is small, reniform, about 200 microns in length and one-half as thick, and of a whitish color. As the embryo develops the egg becomes orange-like in color and the reddish eyes are visible. The duration of the egg stage and different larval instars depends greatly upon temperature conditions. Federov states that a female may

lay as many as 100 eggs. Eddy and Clarke record as high as 14 eggs per day from a single female. The greatest number of eggs laid by a single female in our studies was 22. This, however, undoubtedly does not represent the maximum because the writers took egg-laying records of only a few females.

In securing the length of the different developmental periods, 100 individuals were housed in the screened laboratory, which approximated field conditions, and 50 individuals in each of the constant temperature cabinets, maintained respectively at 25° C. and 30° C. The periods of life of the two feeding larval instars have been consolidated into one unit in tables 1, 2 and 3. These active larvae are somewhat gregarious in habit and tend to conceal themselves in the axils of the leaves of the onion plant. Field counts made at Pleasant Valley during the summers of 1929 and 1930 show that a large percentage of the nymphs had concealed themselves in this portion of the plant at the time the records were taken.

The two more or less quiescent and non-feeding stages are usually found in the soil. As soon as a feeding nymph of the second instar becomes mature it buries itself in the ground, molts and becomes a prepupa. The duration of the prepupa and pupa stages is comparatively short (see tables 1, 2 and 3). No attempt was made to determine accurately the number of generations in a summer in Iowa; records indicate, however, that there may be from 5 to 8. The insect is also commonly found in greenhouses and breeds there throughout the entire year. The adults may be found throughout the winter in favorable hibernating quarters.

DURATION OF DEVELOPMENTAL STAGES (Figs. 2, 3 and 4)

The average time, and the maximum and minimum times, required for the completion of development of the various life stages under the three environmental conditions are shown in tables 1, 2 and 3. Table 4 shows the total time necessary to develop from egg to adult under the three conditions of temperature. In the column marked "theoretical" the figures cited were obtained by combining the minimum (or maximum) time required by any individual within the series to complete the egg stage with the minimum (or maximum) period required by any individual for the nymphal stage and the minimum (or maximum) periods required by any individuals for the prepupal and pupal stages respectively. Such a figure is of interest in that it may show where the most effective temperature range occurs, i. e., the temperature where the actual total time for development most nearly coincides with the theoretical. As seen from the chart, this temperature is at 30° C.

TABLE 1. *In screened laboratory*

| Number of individuals | Time | Stage (length in days) | | | |
|-----------------------|---------|------------------------|-----------------------|---------|------|
| | | Egg | (2 stages) Nymphal | Prepupa | Pupa |
| | Mean | 5.09 | 4.34 | 1.44 | 2.44 |
| 100 | Minimum | 4 | 3 | 1 | 1 |
| | Maximum | 7 | 7 | 3 | 4 |

TABLE 2. *In constant temperature cabinet, 25° ± .1°*

| Number of individuals | Time | Stage (length in days) | | | |
|-----------------------|---------|------------------------|-----------------------|---------|------|
| | | Egg | (2 stages) Nymphal | Prepupa | Pupa |
| | Mean | 6.0 | 6.08 | 1.22 | 2.76 |
| 50 | Minimum | 6 | 5 | 1 | 2 |
| | Maximum | 6 | 7 | 2 | 3 |

TABLE 3. *In constant temperature cabinet, 30° ± .1°.*

| Number of individuals | Time | Stage (length in days) | | | |
|-----------------------|---------|------------------------|-----------------------|---------|------|
| | | Egg | (2 stages) Nymphal | Prepupa | Pupa |
| | Mean | 4 | 4.16 | 1 | 2 |
| 50 | Minimum | 4 | 4 | 1 | 2 |
| | Maximum | 4 | 5 | 1 | 2 |

TABLE 4. *Summary of duration of developmental period in days*

| Temperature | Minimum | | Maximum | | Mean |
|-----------------------|------------------|--------|------------------|--------|-------|
| | Theoret- ical | Actual | Theoret- ical | Actual | |
| In outdoor laboratory | 9 | 11 | 21 | 15 | 13.31 |
| At 25° C. | 14 | 15 | 18 | 18 | 16.06 |
| At 30° C. | 11 | 11 | 12 | 12 | 11.16 |

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PLATE I

Adult of *Thrips tabaci*.

PLATE I



PLATE II

Nymphal stages of *Thrips tabaci*; *a* and *b*, eggs; *c*, first nymphal stage; *d*, second nymphal stage; *e*, prepupa; *f*, pupa.

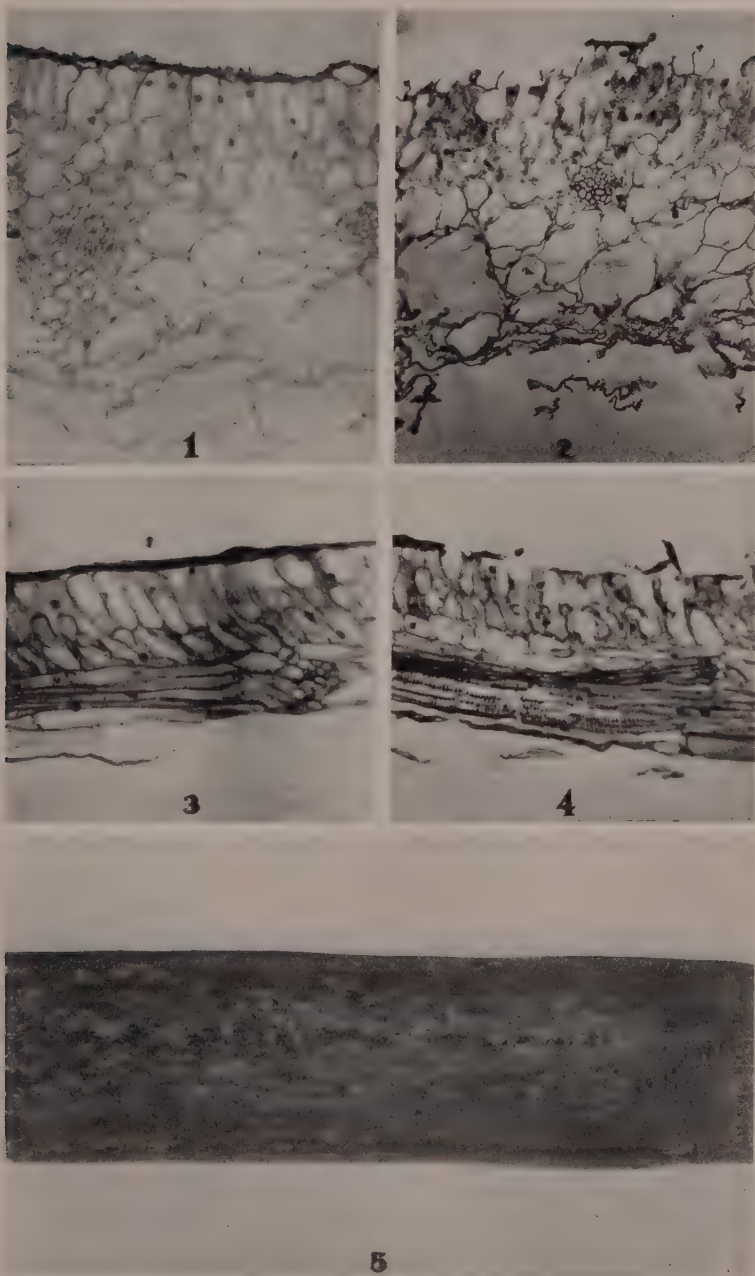
PLATE II



PLATE III

Injuries from feeding activities of *Thrips tabaci*. Figs. 1 and 3, transverse and longitudinal sections, respectively, of normal tissue. Figs 2 and 4, injured plants, transverse and longitudinal sections. Fig. 5, surface view of injury.

PLATE III



A STUDY OF THE NESTING HABITS OF THE RING-NECKED PHEASANT IN NORTHWEST IOWA¹

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This paper presents the data now in hand on one phase of the wildlife research program of Iowa State College²; namely, the study of the nesting habits of the English or ring-necked pheasant (*Phasianus colchicus torquatus* Gmelin).

Although the pheasant is not a native species, little explanation need be made as to the reasons for choosing this bird as an object of study. With the tremendous reduction in the numbers of the native prairie chicken which has accompanied the later stages of the the agricultural development of Iowa, the pheasant, at least in the northern half of the state, has come to assume great importance to the hunting public. So much interest and importance have been attached to this bird that pheasant management has become a major item in Iowa's fish and game program. The reason for an ecological study of the pheasant at once becomes clear.

The region in the vicinity of Ruthven, Iowa, was chosen as the site of the nesting studies for two reasons: first, because a pheasant population thought to be representative is found there, and second, because a field sub-station had already been established and cooperative relations entered into with the farmers and townspeople of the vicinity, in connection with the duck studies carried on by Dr. Paul L. Errington and Mr. Logan J. Bennett.

The general purpose of the work has been to attempt to gain some insight into the ecologic aspects of pheasant nesting, as an aid to the formulation of definite recommendations for pheasant management in Iowa. The present paper deals with the findings to date.

TECHNIQUE

The data embodied in this report are the result of field investigations carried on during the breeding seasons of 1933, 1934 and 1935. During this period, 503 pheasant nests have been under observation, 50 in the summer of 1933, 221 in 1934 and 232 in 1935. The following facts were recorded: number of eggs; approximate date of laying of the first egg of a clutch; date of hatch; number hatched, infertile and dead in shell; if the

¹ Journal Paper No. J291 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 329.

² The Iowa research program 1932-1935 was established at Iowa State College by the College and the Iowa Fish and Game Commission cooperatively, with financial contributions from J. N. ("Ding") Darling. The work was carried on under the administrative supervision of Dr. Carl J. Drake and the direction of Dr. Paul L. Errington.

nest was deserted or destroyed, an attempt was made to establish the cause; identification of cover and lining material, and of the general type of vegetation in which the nest was placed; depth of nest, whether or not roofed over; an evaluation of the efficiency of cover and drainage; exposure; direction faced by nest opening; distance to nearest break in cover or edge of cover; distance to nearest trees and water. Many nests were photographed to make the record more nearly complete. A 20-acre portion of a burned over slough 100 acres in extent was accurately mapped to show the exact location of each nest, and two sketch maps, one of a burned over slough of two and one-half acres, the other of a mowed alfalfa field of the same size, were made for the purpose of illustrating the placing of nests in relation to edges. Censuses of broods were taken each season, in order to obtain data on juvenile mortality. Data from concurrent studies on the food habits of the horned owl, marsh hawk, fox and mink, not included in this paper, have been obtained by the research staff and serve as valuable aids in the consideration of predator losses during the nesting season.

Several methods were employed in the search for nests. By means of articles published in the Ruthven Free Press, through the courtesy of Mr. A. L. Bragg, the editor, and through contacts made directly in the field, the farmers and townspeople were asked to report any nests which they should find. Local cooperators reported 216 nests. Mr. Logan J. Bennett, during the course of the duck studies, reported 21 nests. The remaining 266 nests were found by the author and his wife, frequently assisted by Dr. Paul L. Errington and Miss Ruth Dudgeon, by means of direct search.

Great care was taken throughout the field investigations to avoid causing desertion as a result of human tampering. In general, until the hatch had come off or the nest had been destroyed or deserted, nests were approached to a spot not closer than five to ten feet, the concealing cover was not disturbed nor the eggs touched. In order not to attract the attention of possible predators to the nest, the surrounding cover was not trampled or otherwise changed. Once a nest had been located, it was visited only often enough to insure establishment of the date of hatching, and care was taken not to flush the hen, if she was found on the nest. Of the 503 nests studied, seven desertions have been attributed to the observer. For a further discussion of the technique of nest finding, the reader is referred to Errington (1932).

PLACING OF NESTS

All of the nests studied have been closely associated with the ground level. Some, placed in wind-drifted corn husks, for example, were a few inches above ground level; a few were placed in accidental hollows as deep as six inches. In most cases, the nest was in the form of a scooped out or natural depression, generally varying from one-fourth inch to one and one-half inches in depth, lined with whatever materials were at hand, such as grass, leaves, twigs, dead stalks of older growths. A few pheasant feathers, in one case those of the cock, were often to be seen in the nest, whether as part of the lining or pulled out by accident could not be established. In a few instances, nests were not scooped out, but were placed flat on the ground. Some were on slight elevations, as a tussock in a marsh or other small local area raised a few inches above the surrounding level. There seemed to be no favored direction of exposure or nest

opening, exposures and openings facing all of the points of the compass. Fence rows, roadsides and railroad right-of-ways; alfalfa, sweet clover and small grain fields; sloughs and marshes; pastures and meadows; woods, brush, farmyards, lake shores, ditch banks, gravel pit, waste ground, pot holes, corn fields, plowed ground and stubble served as nesting sites.

The studies made in Iowa indicate that pheasants do not, as a general rule, construct a roof over the nest. Of the 305 nests for which data on this point have been recorded, only two instances of actual roof construction have been noted. Moreover, in the case of one of these—a nest placed in corn husks drifted against a fence—accident may have played a major part, as by the drifting of husks over the back of the incubating bird. A third nest, containing 18 pheasant and 12 quail eggs, had a partially constructed roof, but in view of the known tendency of quail to build a protecting covering over the top of their nests (Stoddard, 1931, p. 25; Errington, 1933, p. 123) it seems more reasonable to suppose that the quail, rather than the pheasant, was the roof builder. The eggs in this nest were not incubated.

Nests were often placed in cover which afforded complete or partial concealment from above, as in tall, thick vegetation, under the fallen dead tops of previous years' growths, or beneath a ball of tumbleweed caught under a fence. Many nests, however, were wholly without overtopping cover of any sort. Plate I, figures A, B and C, are illustrative of the three groups of relative overhead concealment. In Plate I, figure D, is shown an incubating pheasant on a nest with slight overhead concealment.

Table 1 presents the data on the success of nesting attempts in these three groups. It should be pointed out that nests found after the removal of cover by mowing or burning have not been included in the table, as it was not possible in such cases to determine whether or not overhead concealment had been originally present. Because of the exclusion of such nests, the table probably does not represent the proportion of the three groups to all of the nests found. It does, however, show the relation of success to failure of 258 nests in undisturbed cover. The term "success" as applied to nesting attempts, means only that hatching occurred. The analysis of the hatch, concerned with the actual number of hatched eggs, infertile eggs and embryos dead in the shell, is treated in another section.

TABLE 1. *Relation of success to failure among nests wholly, partially, or not at all concealed above*

| Season | Wholly concealed above | | | Partially concealed above | | | Not at all concealed above | | |
|---------|------------------------|-------------|---------------|---------------------------|-------------|---------------|----------------------------|-------------|---------------|
| | No. of nests | No. hatched | Pctg. hatched | No. of nests | No. hatched | Pctg. hatched | No. of nests | No. hatched | Pctg. hatched |
| 1933 | 2 | 0 | 0.0 | 10 | 3 | 30.0 | 7 | 2 | 28.6 |
| 1934 | 46 | 14 | 30.4 | 37 | 9 | 24.3 | 39 | 14 | 35.8 |
| 1935 | 12 | 5 | 41.6 | 72 | 15 | 20.8 | 33 | 5 | 15.2 |
| Average | | | 31.6 | | | 22.7 | | | 26.5 |

At the close of the 1934 season, it seemed that nests in all three groups were about equally successful (Hamerstrom, 1935, p. 11), but the larger volume of data now in hand do not appear to point quite so strongly in that direction.

In addition to recording the relative degree of overhead cover, an estimate of the general concealment of nests was made wherever possible. The terms excellent, good, fair and poor were used, according to the observer's opinion of the concealment value of the nesting cover. Plate I, figures A, B and C, illustrate excellent, good and fair concealment, respectively, according to this system of classification. Table 2 is a summary of the data on 251 nests in undisturbed cover, and shows the percentage of success in cover judged to be excellent, good, fair and poor.

TABLE 2. *Percentage of success in cover judged to be excellent, good, fair and poor*

| Season | Concealment | | | | | | | | | | | |
|---------|-------------|-----|---------|-------|-----|---------|-------|-----|---------|-------|-----|---------|
| | Excellent | | | Good | | | Fair | | | Poor | | |
| | Nests | | Hatched | Nests | | Hatched | Nests | | Hatched | Nests | | Hatched |
| | No. | No. | Pctg. | No. | No. | Pctg. | No. | No. | Pctg. | No. | No. | Pctg. |
| 1933 | 4 | 1 | 25.0 | 10 | 5 | 50.0 | 8 | 3 | 37.5 | 4 | 2 | 50.0 |
| 1934 | 34 | 8 | 23.5 | 26 | 8 | 30.7 | 16 | 4 | 25.0 | 38 | 14 | 36.8 |
| 1935 | 37 | 13 | 35.1 | 25 | 5 | 20.0 | 17 | 2 | 11.8 | 42 | 4 | 9.5 |
| Average | | | 29.3 | | | 29.5 | | | 22.0 | | | 26.4 |

No relation has been observed between the nest site and the distance to trees and water. To illustrate the extremes, nests were found at the bases of trees and as much as 1,300 feet from the nearest tree; and, although Bent (1932, p. 313) states that nests are rarely placed at any great distance from water, they were found at distances from six inches to 865 yards from the nearest available supply. English (1933, p. 16) says, "... the majority of nests were a mile or more from water."

A consistent attempt has been made to evaluate the adequacy of the drainage of nest sites. The terms excellent, good, fair and poor were used to serve as a basis of comparison. A nest on high ground, and so placed as not to be in a puddle of water following a heavy rain, was considered excellently drained, while one in a spot which would collect water, or from which water would not drain away readily, was judged to be poorly placed in this respect. In the latter group were a number of nests, for the most part in sloughs or roadside ditches, placed on ground which in itself offered adequate drainage, but which, because of a rise in water level or the carrying off of a large volume of surface-run-off, would be flooded after even moderate rainfall.

By giving the numerical values 1, 2, 3 and 4 to the four degrees of relative drainage, an average of 2.0, or good drainage, was obtained for 429 nests over the three years of this study. The average figure was not the same for each year, however. In 1933, drainage averaged slightly below good (2.2); in 1934, slightly above fair (2.7); in 1935, half way between

good and excellent (1.5). The low average for 1934 is perhaps accounted for by the drought, because much of the low ground was dry in the spring, offering nesting sites which were flooded by the early summer rains. Such losses are considered under the discussion of flooding as a cause of nest failure.

The tendency of certain gallinaceous birds to place their nests with reference to edges of blocks of cover has been noted by a number of authors. Stoddard (1931, p. 21) states that over 74 per cent of the bob-white nests studied were within 50 feet of roads, paths, edges of fields, or other openings. The Hungarian partridge shows a like preference for edges of cover, according to Yeatter (1934, p. 27), who found half of the

TABLE 3. *Placing of nests in blocks of fairly homogeneous cover*

| Dis- tance from fence or edge | Section One | | | Section Two | | | Section Three | | |
|---|---------------------------------------|--------|--------|----------------|--------|--------|------------------|---------|---------|
| | All data (except Jefferson Slough) | | | Alfalfa (1935) | | | Jefferson Slough | | |
| | Intervals | | | Intervals | | | Intervals | | |
| | No. nests | | Pctg. | No. nests | | Pctg. | No. nests | | Pctg. |
| | 25-ft. | 50-ft. | 50-ft. | 25-ft. | 50-ft. | 50-ft. | 25-ft. | 100-ft. | 100-ft. |
| 1-25 | 39 | | | 16 | | | 2 | | |
| 26-50 | 44 | 83 | 54.2 | 25 | 41 | 54.7 | 4 | | |
| 51-75 | 17 | | | 9 | | | 6 | | |
| 76-100 | 18 | 35 | 22.9 | 12 | 21 | 28.0 | 9 | 21 | 42.9 |
| 101-125 | 9 | | | 3 | | | 1 | | |
| 126-150 | 12 | 21 | 13.7 | 6 | 9 | 12.0 | 3 | | |
| 151-175 | 4 | | | 1 | | | 3 | | |
| 176-200 | 3 | 7 | 4.6 | 2 | 3 | 4.0 | 3 | 10 | 20.4 |
| 201-225 | | | | | | | 1 | | |
| 226-250 | | over | 4.6 | | | | 1 | | |
| 251-275 | | | | | | | 4 | | |
| 276-300 | | | | | | | 2 | 8 | 16.3 |
| 301-325 | | | | | | | 2 | | |
| 326-350 | | | | | | | 2 | | |
| 351-375 | 1 | 1 | | 1 | 1 | 1.3 | 1 | | |
| 376-400 | | | | | | | 1 | 6 | 12.2 |
| to 500 | | | | | | | 2 | 2 | 4.1 |
| to 600 | 2 | 2 | | | | | 2 | 2 | 4.1 |
| to 1500 | 4 | 4 | | | | | | | |
| Total | 153 | 153 | 100.0 | 75 | 75 | 100.0 | 49 | 49 | 100.0 |

hayfield nests in the outer 24 feet of the fields. Examination of English's (1933, p. 16) Michigan figures shows that slightly less than half (46.4 per cent) of the nests in fields were within 50 feet, and two-thirds of the nests within 100 feet, of the nearest fence.

The same apparent preference has been observed in the Iowa studies. Table 3 shows the placing of nests in relatively homogeneous blocks of cover. Nests found in alfalfa, sweet clover, small grains, and grass meadows make up the greater part of the data, with the addition of a few from marshes. As this point concerns blocks of cover, rather than strips, those nests which were found in fencerows, roadsides, railroad right of ways, slough edges, and the like, have not been considered.

To make the analysis more thorough, the table has been divided into three sections. Section one takes in all data except those from Jefferson Slough,³ and includes 153 nests. Seventy-five of these nests, found in alfalfa fields in 1935, have been separated out in section two because of their greater accuracy. These nests were paced off from the nearest fences. In the case of the other nests, distances were estimated. The nests found at Jefferson Slough are treated in section three. These nests were not included in section one because only a part (20 acres) of the 100-acre slough was examined, and this part did not extend quite into the middle. The distances were taken from the accurately made map (fig. 1) which was made of the portion of the slough covered. The edge was considered to be the line between the round stemmed bulrush and the outer zone characterized by *Hordeum jubatum*, *Iris virginica*, *Eupatorium perfoliatum*. This edge was plainly marked on the burned over ground, and showed a rather sharp transition from the heavy cover of the interior of the slough and the lighter, closely grazed periphery.

Figure 1 is the map of Jefferson Slough already referred to, and figure 2 a sketch map of a small alfalfa field, to illustrate the placing of nests with regard to edges.

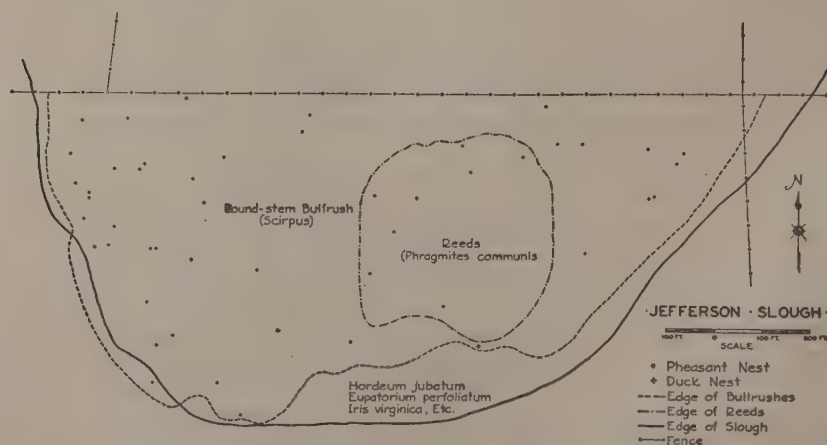


Fig. 1. A map showing the placing of nests found at Jefferson Slough. The map was made after the removal of the cover by fire.

³ Also known as Jemmerston Slough.



Fig. 2. A map showing the placing of nests in a two acre alfalfa field. The map was made after the removal of the cover by mowing.

Sections one and two of table 3 show a concentration of more than one-half of the nests within the first 50 feet, and over three-quarters in the first 100 feet, from the fence or other edge. There is a strong suggestion in section three, however, that the width of the preferred edge zone may be proportional rather than absolute. Among the nests found at Jefferson Slough, the largest share came not in the first 50 feet, but in the first 100 feet. A rough similarity between the nest distribution curves is evident if the alfalfa field nests (section two) are grouped into 50-foot intervals and the Jefferson Slough nests (section three) in 100-foot intervals. This agreement seems all the more significant by the fact that the average of the several alfalfa fields in section two is 7.9 acres and the area of the examined part of Jefferson Slough which lies within the edge boundary is 16.7 acres.

In other words, there seems to be at least a possibility that some proportion of the depth of a field, rather than an absolute strip 50 or 100 feet in depth, may offer the most preferred nesting cover. It may not be stretching the analogy too far to liken this tendency to the human custom of placing a house near the edge of a small block of land, and farther back in a larger one.

NUMBER OF EGGS PER CLUTCH

In the determination of size of clutch, the data used were those from nests in which incubation was known to have been begun, in order to exclude partial clutches which were destroyed or deserted before completion. So far as possible, clutches from which a few eggs had been removed by predators have also been discarded. The averages for the three seasons were: 1933, 12.3 eggs, range 8 to 17; 1934, 10.1 eggs, range 4 to 20; 1935, 12.4, range 8 to 26; average for three seasons 11.2, range 4 to 26. The Michigan pheasant studies also show a variation in average size of

clutch with different seasons. English (1933, p. 7) gives the following figures: 1932, 11.8 eggs, and 1933, 10.9 eggs. Wight (1930, p. 224) adds an average for a third Michigan season, 11.3. Concerning *P. colchicus torquatus* in its native country, Beebe (1922, vol. III, p. 123) says, "Young hens may lay fewer eggs than those a year or two older, but the general number runs from 6 to 12."

Clutches which were considered to represent so-called "dump nests," or the "compound sets" described by Stoddard for bob-whites (1931, p. 28), were found upon several occasions. The largest of these contained 31 eggs, which were not incubated. Single eggs, apparently dropped at random, were frequently observed, particularly during the early spring. This tendency in bob-whites is mentioned by Stoddard (1931, p. 28), who considers such eggs to be indicative of nest destruction, or to occur because the hen could not reach her nest in time. Errington and Bennett (1934, p. 254) commented upon the same carelessness with early duck eggs. Indeed, according to Leopold (1933, p. 366), "Single eggs are sometimes dropped at random by all game birds."

Closely related, perhaps, to the random dropping of single eggs is the laying of one to several eggs in the nests of other ground nesting species, and vice versa. Pheasant eggs were found in partridge nests, duck nests, and in the nest of a king rail. In at least two instances, a single hatched pheasant egg was found in a clutch of hatched blue-wing teal eggs. On the other hand, both quail and partridge eggs were found in pheasant nests. One pheasant nest was taken over by a domestic goose, and another by a domestic chicken.

During all three seasons it was found that clutches became smaller as the season advanced. Table 4 presents a tabular summary, by months, of this shrinkage in clutch size.

The same fact has been observed in the case of other gallinaceous birds. A difference between early and late bob-white clutches is recorded by Stoddard (1931, p. 28), who attributes the difference to the "compound sets, which are found mainly before nesting cover becomes dense." Er-

TABLE 4. Decline in average size of clutch with advance of nesting season

| Laying began in | 1933 | | 1934 | | 1935 | |
|--------------------|--------------------|-----------------------|--------------------|-----------------------|--------------------|-----------------------|
| | Number of nests | Av. size of clutch | Number of nests | Av. size of clutch | Number of nests | Av. size of clutch |
| April | 3 | 13.3 | 7 | 12.6 | 6 | 18.8 |
| May | 10 | 12.5 | 20 | 10.9 | 21 | 12.3 |
| June | 2 | 10.0 | 20 | 9.0 | 11 | 9.3 |
| July | | | 6 | 8.3 | 2 | 10.5 |
| August | | | | | | |
| September | | | 1 | 8.0 | | |
| Total | 15 | | 54 | | 40 | |
| Av. for season | | 12.3 | | 10.1 | | 12.4 |

rington (1933, p. 125), however, speaks of the decrease as being progressive for northern bob-whites. The Hungarian partridge, according to Yeatter (1934, p. 32), shows a similar decrease in size of clutch with the advance of the nesting season.

During the 1935 season, three clutches were found in which some of the eggs were distinctly under normal size. One of these nests had been destroyed before it was found. In the case of the second, containing six small and two normal eggs, four of the small eggs hatched, one chick was dead at about 19 days, with the head at the wrong end of the shell, and one was dead at about 11 days. Neither of the normal sized eggs hatched, one being infertile, the other having developed only three days. The third clutch contained one small egg among 20 of normal size. The small egg was fertile but unembryonated, although 19 of the remaining 20 hatched.

FERTILITY, VIABILITY AND HATCH

It has been the practice, throughout this investigation, to open eggs remaining after the hatch or desertion of a clutch, in order to determine the number of eggs which were infertile or in which the embryo had not fully developed. In several cases one or more eggs were removed from the nest by unknown agencies before the hatch, and in a few instances the eggs remaining after the hatch were not examined. Table 5, based upon 64 hatched clutches for which the data are complete, plus one for which all of the eggs were known to be infertile, summarizes the data on fertility, viability, and hatch.

Thirty-eight, or 58.4 per cent, of the 65 clutches in table 5 were made up entirely of fertile eggs. In only 24, or 36.9 per cent, however, did every egg hatch. Embryos dead in the shell varied from 0 to 62.5 per cent per clutch. In general, while fertility averaged over 90 per cent, wholly successful clutches were not the rule, and most clutches contained one to several chicks which died before hatching.

It was suspected, when only the data from 1933 and 1934 were available (Hamerstrom, 1935, p. 19), that the higher percentage of dead embryos in 1934 might be attributable to drought conditions. The season of 1935 was somewhat wetter than normal, and showed a still higher loss. Until more information is at hand for normal years, however, it seems best not to make definite conclusions on this score.

Two cases of distinctly abnormal development were encountered among the embryos found dead in the shell. In both, development had

TABLE 5. *Tabular summary of fertility, viability and hatch*

| Season | Number | | Percentage | | |
|---------|----------|------|------------|---------------|---------|
| | Clutches | Eggs | Fertile | Dead in shell | Hatched |
| 1933 | 11 | 136 | 91.2 | 1.5 | 89.7 |
| 1934 | 32 | 329 | 92.7 | 12.2 | 80.5 |
| 1935 | 21 | 258 | 94.6 | 14.0 | 80.6 |
| Average | | | 93.1 | 10.8 | 82.3 |

progressed to about the twenty-first day, stopping approximately at the point of inclusion of the yolk sac. One, which was preserved, has four legs, four wings, two sets of cervical vertebrae, one eye, and a laterally crossed bill. The second had a deformed mandible.

COVER, DATES OF NESTING AND SUCCESS OF NESTING ATTEMPTS

Nests were found in a wide variety of cover types, but as it was not considered feasible to restrict the nest hunting to a circumscribed area, the relative number of nests for each type could not be determined. As an example of the former type of investigation, the reader is referred to English (1933). The following discussion of nesting cover is based upon cover types in which nests were found at different periods of spring and summer, and, as cover and laying dates are closely related, the two aspects of the problem are here considered together.

Figures 3 and 4 are graphs showing the number of clutches begun during ten-day intervals in 1934 and 1935, respectively. The dotted line represents the most accurate data, from 62 nests in 1934 and 65 nests in

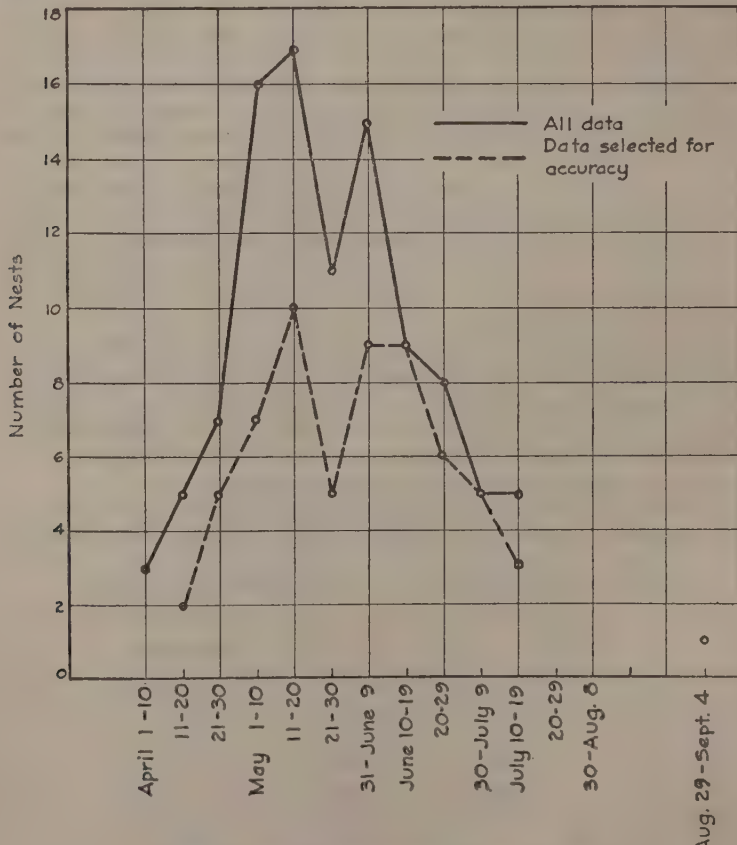


Fig. 3. Dates of Beginning of Clutch—1934

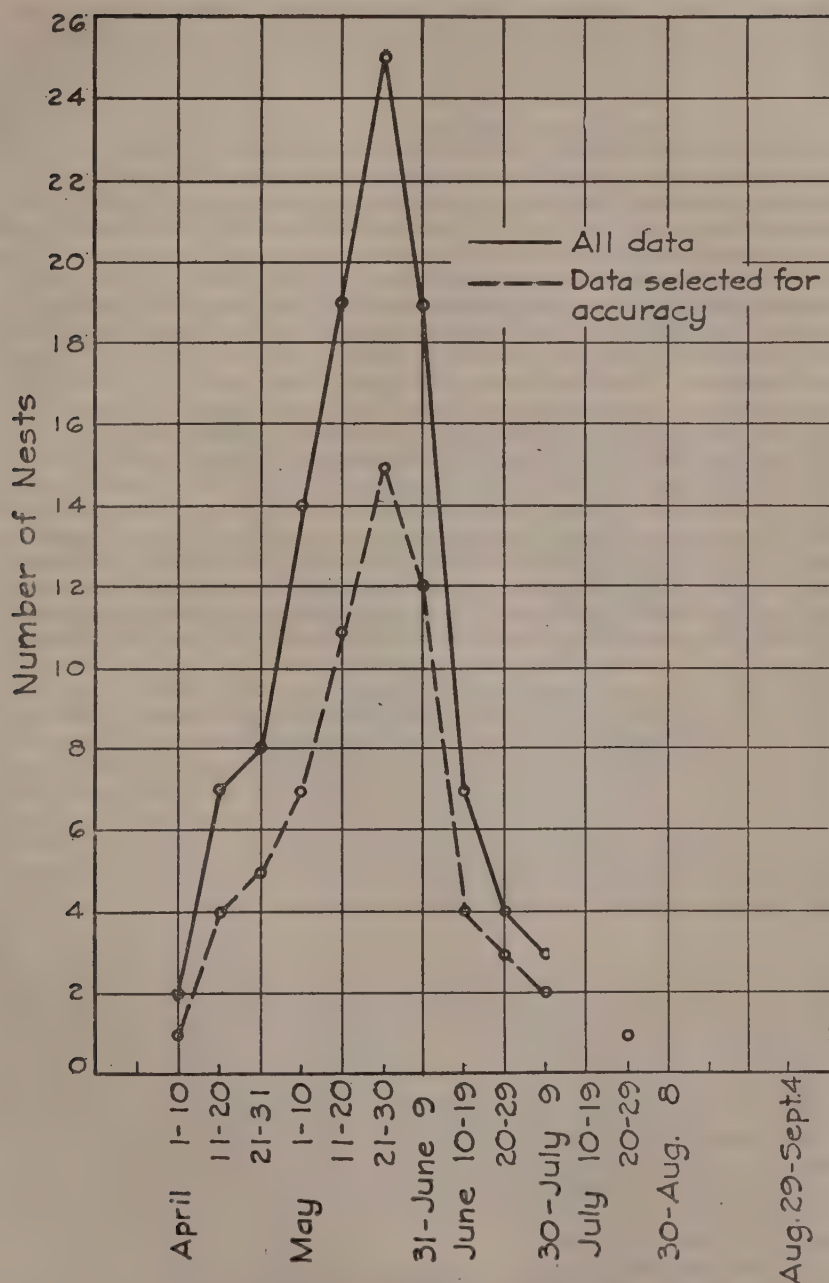


Fig. 4. Dates of Beginning of Clutch—1935

1935; the solid line, all data on this point, 102 nests in 1934 and 109 nests in 1935. For the remaining nests it was impossible to establish the date of laying of the first egg. The data from 1933 are too scanty to permit graphing.

Comparison of the two graphs shows a double peak in the nesting curve for 1934, and a single peak in 1935. In the absence of sufficient data from normal years, it would perhaps be premature to make definite statements at this time, but it may be suggested that the trough in the 1934 curve may be attributed to the drought of that season, which was broken, in the region covered by this study, in early June.

The earliest recorded laying dates were: 1933, April 25; 1934, before April 13; 1935, April 4. The latest recorded dates for the beginning of a clutch were: 1933, between June 25 and July 2; 1934, September 4; 1935, July 21. These figures show a nesting season extending from early April into September. An examination of the graphs, however, will indicate that the majority of nests were begun during April and May, in 1935, and April, May and early June in the probably less typical season of 1934.

English (1933, p. 40) finds that "the majority of successful clutches were laid in April and May." Nest hunting in Iowa was discontinued in early August of all three seasons, because the few nests found after late July did not repay the time spent in searching for them. That nesting straggles on through July, August and September is indicated by Wight's statement (1930, p. 224), "Nesting occurred over a period of at least five months, April to September, inclusive," by occasional reports, late in the season, that broods no more than a few days old had been seen, and by the record of a nest known to have been begun in September.

A discussion by months of the types of cover in which nests were found is hampered by the fact that vegetative growth is not governed by calendar dates, but may vary by as much as several weeks from season to season. As pointed out by Leopold (1933, p. 165), a strictly phenological table would be of more accuracy than an adherence to months and dates in the examination of nesting data. Although the first two seasons differed considerably from the last in precipitation, temperature and correlated plant growth, some generalizations may be made concerning the phenology of pheasant nesting, while still holding to a monthly basis of comparison.

Early spring cover was almost entirely confined to the dead tops of the vegetation of preceding years, and included such cover plants as grasses and sedges; stalks of goldenrod, aster, sunflower, sweet clover, and other fairly stout-stemmed herbaceous plants; wind-drifted corn husks and leaves of trees, and the like. April nests were found almost exclusively in such material, which was available, if not previously removed by grazing, mowing and burning, in the following cover types: fencerows, roadsides, railroad right of ways, slough edges, stubble fields, woodlots, lake shores, gravel pits and other odd corners, and farmyards. The concealment afforded these early nests by the cover in which they were placed varied from excellent to none at all. A few nests were found on absolutely bare fall-plowed fields, when good cover was available within a hundred yards or so.

May brings a tremendous increase in available cover. The new growth of grasses, sedges and other herbaceous plants adds to the value of the cover types already in use and offers new locations, and the appear-

ance of alfalfa introduces a new and very attractive type of cover. Pastures, generally without much cover value in early spring because of too close grazing during the preceding fall, in late April and early May begin to contribute suitable nesting sites. The leafing out of brushy species during the same period offers a new cover type, but one which, on the basis of evidence to date, is not utilized to any great extent.

In June, in addition to the preceding cover types, fields of small grains become available. Upon three occasions, at least, cornfields were used for nesting before the final cultivation. The first mowing of alfalfa generally occurs during this month, resulting in the destruction of many nests and the removal of a large area of nesting cover. Some of the nests made in the small grains at this time probably represent renesting attempts following such destruction, although Wight (1930, p. 223) has evidence, based upon examination of ovaries, that some hens regularly nest much later than others.

During July, no new cover type of major importance appears, but the month has importance from the standpoint of cover in that the second cutting of alfalfa and the harvesting of small grains interrupts nesting again. There may also be a considerable amount of mowing in the grass meadows and sloughs. The interval between the time at which the second growth of alfalfa is suitable for nesting and the second cutting is generally too short to permit the hatching of a brood, but nests begun early enough in the small grains have a good chance to hatch before the harvest. Nests begun later, however, may be destroyed.

The foregoing consideration of cover types has been admittedly very general. A complete list of the plant species involved would be of little interest, but a review of the more important species may have some value. In a few instances, such as oats and alfalfa, the common names were considered sufficiently diagnostic, but the author's knowledge of systematic botany was not always adequate to identify to the species plants not in bloom, grazed down or otherwise injured, dead, or those belonging in particularly difficult genera.

The most convenient method of presentation is according to the situations in which nests were found. For the sake of brevity, these locations will be grouped into several major types, with a discussion of the most important plant species of each.

Sloughs, marshes and pot holes, because of the essential similarity of the plant associations in the parts utilized for nesting cover, are considered to belong in one grouping. In general, only the edges were found to be dry enough for nesting purposes. The vegetation of this peripheral as-sociates is quite definite, in most cases, and is characterized by: grasses—*Hordeum jubatum*, *Calamagrostis canadensis*, *C. inexpansa*, *Poa*, spp., and patches of *Spartina pectinata*; sedges—*Carex* spp., and probably others; such herbaceous plants as *Acorus Calamus*, *Iris virginica*, *Stachys palustris*, *Lycopus rubellus*, *L. americanus*, *Mentha canadensis* var. *arvensis*, and other mints, *Ambrosia artemisiifolia*, *Eupatorium perfoliatum*, and in dryer locations, *Solidago canadensis* and other goldenrods, *Aster* sp. Wil-lows are sometimes found in conjunction with slough edges. In one instance, at Jefferson Slough, the entire area of the slough was dry, and nests were found in the round-stemmed bulrush (*Scirpus* spp.) and reed (*Phragmites communis*) rather than in the edge zone.

At the close of the 1934 season, more nests, on a per acre basis, had been found in the slough, marsh and pot hole type of cover than in any other. It was believed at that time (Hamerstrom, 1935, p. 25) that a preference for such cover was indicated. While this type of cover in 1934 gave the best returns for time spent in nest hunting, in 1935 it gave the least. Careful search of the very sloughs which had been so productive the season before yielded but a very few nests. Although the latter season was much wetter than the former, the edge zone appeared to be no less available for nesting, and to human eyes, at least, seemed to offer even better cover. It may be that pheasants prefer other cover or higher ground, but were forced into the sloughs in 1934 because there were few other places offering adequate concealment. It is quite plain that in the season of 1934, through drought and the resultant grazing down of almost every spear of vegetation, there was very little cover along roadsides and fencerows and in other locations. In 1935, however, there was an abundance of cover everywhere, and a definitely greater number of situations from which to choose.

In this type of cover 147 nests have been under observation, 142 in sloughs and marshes and 5 in pot holes. In calculating the percentage of success for nests in this type of cover, 51 nests found at Jefferson Slough, Dickinson County, have been excluded. This slough, in a vicinity not regularly covered in the nest studies, was visited only after the cover had been removed by fire, for the purpose of mapping the nests so exposed. As the number of nests destroyed there was so large, and as the incidence of marsh fires in the region under observation was so low, it seems that inclusion of these figures would introduce an error of considerable size. One other slough nest, found in 1933 and not revisited, has also been eliminated, since success or failure was not determined. On the basis of the remaining 95 nests in the slough, marsh and pot hole cover, 17, or 17.8 per cent, were successful. Plate II, figure A, is illustrative of nests in this type of cover.

From the rich variety of vegetation to be found in roadside, fencerow and railroad right of way cover, it is almost impossible to sift out those species which are most characteristic, or which may be said to be of most value as nesting cover. The plants most commonly encountered as major constituents of nest cover were: grasses—*Poa pratensis*, the most frequently used, but also the most commonly encountered in many locations, wind-drifted corn husks, *Spartina pectinata*, *Phleum pratense*, *Agrostis alba*, *Setaria lutescens*, *S. viridis*, *Hordeum jubatum*, *Stipa spartea*, *Elymus* sp., *Eragrostis* sp., *Agropyron smithii*, *A. repens*, *Panicum virgatum*. In many instances, due to the author's lack of familiarity with this group during the first two seasons, no identification was made. Forbs: dead balls of *Amaranthus graecizans*, *Rosa* spp., escaped alfalfa and sweet clover, *Lathyrus venosus*, *Rumex* sp., *Convolvulus sepium*, *Solidago* spp., *Aster* spp., *Lepachys pinnata*—often noted in 1934—and other sunflowers, *Silphium perfoliatum*, *S. laciniatum*, "weeds." In this type of cover 134 nests have been found, 71 along roadsides, 35 in fencerows, and 28 in railroad right of ways. A roadside nest is illustrated by Plate II, figure B.

Percentages of success were: roadside, 27.5 per cent; fencerow, 20.6 per cent; railroad, 35.7 per cent. The data are suggestive that width of cover strip, in this cover type, may be associated with hatching success. Thus, the railroad right of way, the widest of the three, shows the highest

percentage of success; fencerows, which are the narrowest, contained the least number of successful nests, and roadsides, of intermediate width, show intermediate success. As these strips of cover serve as avenues of travel for ground predators, with fenceposts as convenient lookout points for crows and hawks, it might be reasonable to suppose that the wider the strip, the fewer the chances that hen or eggs would be noticed by predators or disturbed by man. It must be admitted, however, that the cover along a railroad right of way, although subject to burning, is less apt to be disturbed than that along a roadside or fencerow.

So much has already been written concerning alfalfa and small grain fields as nesting sites for mid-western game birds that little remains to be said here. It is a matter of common knowledge that most nests placed in fields of alfalfa and sweet clover are destroyed or deserted, with the killing of a number of birds which do not flush in time to avoid the sickle bar, and that the harvesting of the small grains has the same result, although to a lesser extent.

Nests observed in alfalfa fields numbered 111, of which 13.6 per cent hatched. One of these nests was mowed over at the time of hatching. English's figures (1933, p. 29) over a two-year period show that 15.2 per cent of nests in "hayfields" were brought to a successful conclusion. In four cases, among the Iowa nests, a patch of uncut alfalfa was left about the nest, but only one bird returned to hatch her clutch. One of these nests, however, was deserted because of the observer's attentions. Nine birds returned to their nests after they had been cut over; one, under the protection of a forkful of hay, incubated a clutch of infertile eggs for more than 30 days before giving up; another, whose nest was mowed over on June 21, 1934, was observed on the nest through July 10, during which period one of the four eggs of the clutch was destroyed. On July 15 the remaining three eggs were found to have been broken. The eggs in six nests disappeared within a few days after mowing. One of these nine nests hatched. According to the reports of farmers in the fields under observation, eight adults were killed outright by mowing machines, and two were crippled, one of which, having lost both legs, surely died later. Two more hens were struck, but the extent of their injuries could not be determined. An unknown number of juveniles, one from each of two fields, and "several," according to the mower, in another, were killed by mowing in the alfalfa fields under observation.

Nest losses in alfalfa fields have unquestionably been high, totalling 86.4 per cent. It might not be amiss, however, to point out that in all other cover types for which the data are not at all extensive, less conspicuous but nevertheless important losses occur, in every case over 50 per cent. Also, not all of the failures in alfalfa were caused by mowing. In 1935, for example, at least 11, or 14.0 per cent, of the 79 nests found in alfalfa were known to have been deserted before mowing was begun.

Five nests, one of which hatched, were found in sweet clover fields. A strip of uncut clover was left around one nest, and the rest of the field plowed under. Although the hen returned to her nest, the eggs disappeared within a few days. One bird was struck by the mowing machine.

It appears at present that small grain fields are utilized for nesting to a lesser extent than is generally supposed. As but few nests were found, a particular effort was made to obtain data on this point. Fields totalling 130 acres were carefully combed for nests, resulting in the finding of but

three by this method. Cooperators of proved interest and ability were asked to watch carefully during the cutting and shocking, and to mark the site of any nests found. By this means three nests in 120 acres were discovered. Other farmers, in fields totalling 265 acres, were specifically asked to report any nests, no matter how few, but reported none. A general request for information was published each week in the Ruthven Free Press, and was productive of thirteen. In view of the willingness to cooperate which was shown by the farmers of the vicinity, it would seem that there actually were not many nests in the small grains.

Complete data are not at hand for two of the 19 nests in this type of cover. Of the remaining 17, 8, or 47.1 per cent, hatched. Four birds returned after the cutting, one to hatch her clutch, and one to lose her eggs a few at a time. The other two nests were not revisited. Two hens were struck by the reaper.

Most slough edges in the region under observation are pastured; hence, most slough edge nests are, strictly speaking, pasture nests. However, because of the distinct differences between the vegetation of slough and marsh edges and the higher meadows farther back, where such exist, the term "pasture" is here used to refer only to the grassy portion and to pastures not associated with sloughs and marshes. By the same token, "pasture" is actually grass meadow, but again it is advisable to employ differentiating terms, this time to distinguish between meadows which are grazed and those cut for hay. "Grass meadow" refers to the latter situation, and includes one or two nests in meadows which were not cut. Plate II, figure C, illustrates a grass meadow nest.

Twenty-three nests were found in grassy locations of the pasture and grass meadow type, 6 in pastures, 17 in meadows. These nests were placed in the following cover: *Poa pratensis*, *Setaria lutescens*, *S. viridis*, *Phleum pratense*, *Agrostis alba*, *Agropyron pauciflorum*, *Hordeum jubatum*, *Ambrosia artemisiifolia*, *Solidago* spp., in various combinations. Seven, or 30.4 per cent, of these nests hatched, 5 in a pasture, 2 in a meadow. After the mowing, one hen returned to lay another egg, but the entire clutch subsequently disappeared. Two were struck by the mowing machine.

Pheasants do nest in woods, as attested by Errington (1923), who found eight nests in three days while inspecting tree claims in Moody County, South Dakota; by Wight (1930, p. 223), English (1933, p. 18) and the twelve woods nests found during the Iowa studies. On the other hand, judging by the number of wooded areas in which careful search disclosed no nests, the author is inclined to agree with Wight's statement (1933, p. 7) that pheasants find other locations more attractive.

The type and effectiveness of cover varied greatly among the Iowa woods nests. Two were placed on ground which had no understory of vegetation in which to hide the nest. The concealment of the others was afforded by *Smnphoricarpus occidentalis*, *Ribes americanum*, tree seedlings, trimmed and fallen branches, *Elymus* sp., *Poa pratensis*, *Hydrophyllum virginianum*; and less frequently *Nepeta Cataria*, *Verbascum Thapsus*, *Galium* sp., *Chenopodium* sp. Nests were found in several sorts of wooded areas. Four were in woodlots made up of various deciduous trees, including one largely of oak (*Quercus* spp.), three in mixed maple (*Acer* sp.) box elder (*Acer negundo*), linden (*Tilia americana*), and cottonwood (*Populus deltoides*). Three were in oak-hickory (*Quercus* spp.-*Carya ovata*) woods associated with sloughs; two on an oak-hickory

(*Quercus* spp.-*Carya ovata*) hillside with a fair interspersation of maple (*Acer nigrum*), linden (*Tilia americana*), and ironwood (*Ostrya virginiana*); one each in river bottom woods, made up largely of elm (*Ulmus americana*) and ash (*Fraxinus lanceolata*), and a wild plum (*Prunus* sp.) and haw (*Crataegus* sp.) thicket. One nest, found in an apple orchard, has been included with the woods nests. Five, or 41.7 per cent, of the woods nests hatched.

Comparatively few brushy areas are to be found in the part of Iowa in which this study has been conducted, and this type of cover also has been used but little by nesting pheasants. Ten nests were found in brushy cover, not including two woods nests which were also associated with brush. In the case of seven, *Symphoricarpos occidentalis* was the major cover species, with an understory of *Poa pratensis*. One of these nests was concealed by *Ribes americanum* as well. Brushpiles, one in a patch of *Rhus glabra*, harbored two nests. The tenth was in a mixture of *Rubus* sp., *Symphoricarpos occidentalis*, *Muhlenbergia* sp., leaves and twigs. Two nests, or 20.0 per cent, hatched. Plate II, figure D, is a photograph of a nest in brushy cover.

Forty-one nests were observed in locations which were considered, on the basis of the number of examples of each, to be of a miscellaneous nature. Further work may remove any or all of these locations from the present grouping. Ditch banks, for example, perhaps should be classed with roadsides, fencerows and railroad right of ways, as all four are rather similar in cover characteristics; but as only three nests have been found to date along ditches, judgment is suspended. Cover for these nests was *Poa pratensis*, corn husks, dead stalks of *Xanthium* sp. and *Ambrosia trifida*. None of these nests hatched. Farmyards were chosen as the sites of eight nests. As all of these were reported by the landholders, it may be that most of the nests so placed were discovered, indicating a low relative importance; or, as four of these nests were begun in April and two before the middle of May, it may be that farmyards represent an important item in the spring cover. Cover utilized was: unidentified grass, *Oxybaphus nyctagineus*, *Amaranthus* sp., *Arctium* sp., *Ambrosia trifida*, cultivated strawberry, raspberry, rhubarb and gooseberry. Two, or 25.0 per cent, hatched.

Wasteland, because there is so little of it in northwest Iowa, is placed in the miscellaneous category. Five nests were found in odd corners, placed in *Poa pratensis*, *Stipa spartea*, *Andropogon* sp., other grasses, *Thalictrum* sp., *Spiraea* sp., *Solidago* sp., *Ambrosia trifida*, *Lactuca* sp., escaped alfalfa and weeds. One, or 20.0 per cent, hatched.

Four nests were found in gravel pits. Three of these were beneath dead sweet clover tangles, one in a thin stand of *Poa pratensis*. One, or 25.0 per cent, hatched. Four nests had been made along lake shores, in *Poa* sp. and weeds, dead stalks of *Scirpus fluvilatilil*, *Poa pratensis* and *Bromus tectorum*, dead sweet clover, *Solidago* sp. One, or 25.0 per cent, hatched. One nest, in dead stalks of *Ambrosia trifida* along a creek bank, did not hatch.

A few nests were reported in stubble fields. Two of these were in corn stubble, *Setaria lutescens* and *S. viridis*, two in sweet clover stubble, and one in alfalfa stubble. Only one hatched (20.0 per cent), and that after half of the eggs had been broken and every vestige of cover removed

by cultivating. In 1934, three nests were reported by farmers who had found them while cultivating corn. Whether this was another reflection of the general shortage of suitable cover which was so evident that season, or whether a few nests are regularly placed in cornfields cannot be stated at this time. All three nests were at the base of hills of corn. One was hidden only by the growing corn, which leaned over it; two were between the corn and plants a few inches away, in one case *Asclepias* sp., in the other, *Polygonum coccinea*. All three hatched. Five nests were placed on fall-plowed ground without any concealing cover. One, or 20.0 per cent, hatched. Three nests, of which two (66.6 per cent) hatched, were reported from unspecified situations. Cover for these nests was: *Poa* sp., *Elymus* sp.

Although the relative number of nests from each type of cover has not been determined, the summary (table 6) of the data on success of nesting attempts in the various types may have some value. With the exception of the 51 nests at Jefferson Slough, for reasons already noted, and the 7 nests for which the data are incomplete, all of the nests encountered in the study are included in this table.

In order to make the exposition as complete as possible, a similar table (table 7) has been prepared for live nests only; that is, only those nests in which egg laying or incubation was known to have been going on during the period of observation. Since it was not known how many nests in alfalfa, sweet clover and small grains were live nests at the time of cutting, all figures from these cover types have been excluded.

It will be noticed that in neither table is there any cover type, for which data are extensive, which showed a very high percentage of success.

According to these figures, 342, or 76.9 per cent, of the nesting attempts summarized in table 6 were unsuccessful. While this figure is not absolutely representative for the region, since the investigation was conducted on a quantitative basis, rather than consisting in the combing of a definite area, the high rate of failure for each of the important types of nesting cover indicates that it is a fairly close approximation of the situation. It is possible that nests found by the author would be those most easily found by predators, and therefore those most subject to disturbance. The relatively low loss attributed to predators, and the thoroughness of the nest hunting methods, however, may indicate that such is not the case.

In attempting to evaluate causes of nest destruction or desertion, greatest caution has been observed. Where the evidence was not clear, it was preferred to designate cause of failure as "unexplained," rather than to make poorly founded judgment. A consistent effort has been made to differentiate between primary and secondary nest disturbance, and to deal only with the former.

More nest failures have been traced directly to man's activities than to any other single agency. The destruction of 179, or 40.2 per cent, of the nests under observation were accounted for by man and his agricultural practices. Even if the 62 failures which could not be diagnosed were laid to any other single agent, or were lumped with general predation, man would still head the list. Losses caused by man, which comprise 52.3 per cent of all failures, may be sub-divided into several groups.

One hundred two nests were destroyed or caused to be abandoned by mowing; namely, 82, or 74.5 per cent, of the nests placed in alfalfa; 4, or

TABLE 6. *Success of nesting attempts in different types of cover—all nests*

| Cover type | Number of nests | | | | Number hatched | | | | Percentage hatched | | | |
|----------------------------|-----------------|------|------|-------|----------------|------|------|-------|--------------------|-------|------|-------|
| | 1933 | 1934 | 1935 | Total | 1933 | 1934 | 1935 | Total | 1933 | 1934 | 1935 | Ave. |
| Slough, Marsh and Pot hole | 3 | 73 | 19 | 95 | 2 | 11 | 4 | 17 | 66.6 | 15.1 | 21.1 | 17.8 |
| Roadside | 14 | 16 | 39 | 69 | 3 | 7 | 9 | 19 | 21.4 | 43.8 | 23.1 | 27.5 |
| Fencerow | 3 | 11 | 20 | 34 | 1 | 3 | 3 | 7 | 33.3 | 27.3 | 15.0 | 20.6 |
| R.R. right of way | 2 | 6 | 20 | 28 | 2 | 2 | 6 | 10 | 100.0 | 33.3 | 30.0 | 35.7 |
| Alfalfa | 9 | 22 | 79 | 110 | 2 | 5 | 8 | 15 | 22.2 | 22.7 | 10.1 | 13.6 |
| Sweet cl'v'r | 0 | 4 | 2 | 6 | — | 0 | 1 | 1 | — | 0.0 | 50.0 | 16.7 |
| Small Gr's | 0 | 9 | 8 | 17 | — | 3 | 5 | 8 | — | 33.3 | 62.5 | 47.1 |
| Pasture and Grass Meadow | 2 | 7 | 14 | 23 | 0 | 2 | 5 | 7 | 0.0 | 28.6 | 35.7 | 30.4 |
| Woods | 2 | 4 | 6 | 12 | 2 | 2 | 1 | 5 | 100.0 | 50.0 | 16.7 | 41.7 |
| Brush | 1 | 5 | 4 | 10 | 0 | 2 | 0 | 2 | 0.0 | 40.0 | 0.0 | 20.0 |
| Misc. | | | | | | | | | | | | |
| Farmyard | 0 | 4 | 4 | 8 | — | 1 | 1 | 2 | — | 25.0 | 25.0 | 25.0 |
| Wasteland | 3 | 0 | 2 | 5 | 1 | — | 0 | 1 | 33.3 | — | 0.0 | 20.0 |
| Stubble | 0 | 1 | 4 | 5 | — | 1 | 0 | 1 | — | 100.0 | 0.0 | 20.0 |
| Plowed fld | 0 | 0 | 5 | 5 | — | — | 1 | 1 | — | — | 20.0 | 20.0 |
| Lake shore | 1 | 2 | 1 | 4 | 0 | 1 | 0 | 1 | 0.0 | 50.0 | 0.0 | 25.0 |
| Gravel pit | 1 | 0 | 3 | 4 | 1 | — | 0 | 1 | 100.0 | — | 0.0 | 25.0 |
| Cornfield | 0 | 3 | 0 | 3 | — | 3 | — | 3 | — | 100.0 | — | 100.0 |
| Ditch bank | 1 | 1 | 1 | 3 | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Unspecified | 2 | 1 | 0 | 3 | 1 | 1 | — | 2 | 50.0 | 100.0 | — | 66.7 |
| Creek bank | 0 | 0 | 1 | 1 | — | — | 0 | 0 | — | — | 0.0 | 0.0 |
| All Nests | 44 | 169 | 232 | 445 | 15 | 44 | 44 | 103 | 34.1 | 26.0 | 19.0 | 23.1 |
| Live Nests | 17 | 54 | 68 | 139 | 5 | 22 | 25 | 52 | 29.4 | 40.7 | 36.7 | 37.4 |

80.0 per cent, of the nests in sweet clover; 7, or 41.2 per cent, of the nests in grass meadows; 2, or 2.1 per cent, of those in sloughs; 4, or 5.8 per cent, of those along roadsides; 2, or 5.8 per cent, of fencerow nests; 1, or 20.0 per cent, of those in waste ground. Eleven hens were known to have been killed and three injured by the mowing machine in connection with the destruction of these nests. Instances of pheasants returning to their nests after the cutting of alfalfa have already been referred to. Similarly, six hens returned to roadside nests which had been mowed over; four of these hatched, and the outcome of one is unknown.

The harvest caused the failure of 9, or 52.9 per cent, of the nests observed in small grain fields. One incubating pheasant was killed by the reaper. In the absence of more extensive data on nests in this type of cover, it might be of value to quote English (1933, p. 29): "Only rarely would a pheasant nest be destroyed in a grain field by the actual process of harvesting the grain, because by this time most clutches have come off safely."

TABLE 7. *Success of nesting attempts in different types of cover—live nests only*

| Cover Type | Number of Nests | | | | Number Hatched | | | | Pctg. Hatched |
|----------------------------|-----------------|------|------|--------|----------------|------|------|--------|---------------|
| | 1933 | 1934 | 1935 | To-tal | 1933 | 1934 | 1935 | To-tal | 1933-35 |
| Slough, Marsh and Pot hole | 2 | 26 | 7 | 35 | 1 | 6 | 3 | 10 | 28.6 |
| Roadside | 7 | 8 | 20 | 35 | 1 | 5 | 8 | 14 | 40.0 |
| Fencerow | 1 | 4 | 9 | 14 | 0 | 2 | 3 | 5 | 35.7 |
| Railroad right of way | 0 | 2 | 12 | 14 | — | 0 | 6 | 6 | 42.9 |
| Alfalfa | | | | | | | | | |
| Sweet Clover | | | | | | | | | |
| Small Grains | | | | | | | | | |
| Pasture and Grass Meadow | 2 | 4 | 3 | 9 | 0 | 1 | 2 | 3 | 33.3 |
| Woods | 0 | 0 | 3 | 3 | — | — | 1 | 1 | 33.3 |
| Brush | 0 | 2 | 2 | 4 | — | 2 | 0 | 2 | 50.0 |
| Miscellaneous | | | | | | | | | |
| Farmyard | 0 | 3 | 3 | 6 | — | 1 | 1 | 2 | 33.3 |
| Wasteland | 1 | 0 | 1 | 2 | 1 | — | 0 | 1 | 50.0 |
| Stubble | 0 | 1 | 0 | 1 | — | 1 | — | 1 | 100.0 |
| Plowed field | 0 | 0 | 4 | 4 | — | — | 1 | 1 | 25.0 |
| Lake shore | 1 | 1 | 0 | 2 | 0 | 1 | — | 1 | 50.0 |
| Gravel pit | 1 | 0 | 2 | 3 | 1 | — | 0 | 1 | 33.3 |
| Cornfield | 0 | 3 | 0 | 3 | — | 3 | — | 3 | 100.0 |
| Ditch bank | 0 | 0 | 1 | 1 | — | — | 0 | 0 | 0.0 |
| Unspecified | 2 | 0 | 0 | 2 | 1 | — | — | 1 | 50.0 |
| Creek bank | 0 | 0 | 1 | 1 | — | — | 0 | 0 | 0.0 |
| Total—Number | 17 | 54 | 68 | 139 | 5 | 22 | 25 | 52 | 37.4 |
| Average—Percentage | | | | | 29.4 | 40.7 | 36.7 | 37.4 | |

Fire destroyed 13 nests, all in slough cover, in the region included in this investigation. These nests represent 13.7 per cent of the nests placed in sloughs, and were all destroyed as the result of carelessness. One cock pheasant, one cottontail, a toad, and many turtles were killed in this fire. The burning of Jefferson Slough, although not considered in the quantitative data, is illustrative of the damage to wildlife caused by uncontrolled burning. The whole slough, 100 acres in extent, was burned over by a fire which got out of hand nearby. Only 20 acres were examined, as the work was brought to a conclusion by a heavy rain which flooded the area. The toll recorded was: destruction of 51 pheasant nests and one teal nest, killing of four pheasants, one duck (teal?), one muskrat and one small bird which could not be identified.

Domestic animals have had a fair share in the destruction of nests, accounting for 28. The practice of turning out cattle and sheep to graze on almost every piece of ground, including roadsides and fencerows, not otherwise directly utilized in farming operations resulted in the failure of 24 nests. The most usual cause of failure was the removal of nest cover,

thus exposing nests to whatever egg hunters should happen to pass by. Occasionally, eggs were broken by trampling. Seven nests, deserted because of grazing or trampling, were placed in slough cover, 7 along roadsides, 5 in fencerows, one each in alfalfa, pasture, lake shore sedges, railroad right of way, and orchard. Hogs, rooting in a woodlot, destroyed one nest; a domestic goose took over one nest in an alfalfa field; a farmer's dog ate the eggs in one alfalfa field nest; and one bird was driven from her farmyard nest by a house cat.

The major items above do not exhaust the list of man's destructive influences. Nest failures were brought about by such activities as: plowing, 4; cultivating, 1; dock digging along roadsides, 2; cutting thistle in a pasture, 1; blasting and woodcutting, 1; two nests, one in a slough and one in a fencerow, were stepped in; one was placed too close to a summer cottage; in 5 cases, nesting birds were disturbed too many times by curious people; one hen was caused to desert by the burning of a large pile of brush within a few feet of her nest. Desertions of 7 nests were traced to the author: 3 in slough cover; one each in a pot hole, roadside, railroad right of way; one, before mowing, in an alfalfa field. Two clutches were taken by farmers and placed under domestic chickens.

To predators may be accounted the loss of 66 nests, representing 14.8 per cent of the nests regularly under observation, or 19.3 per cent of all failures. Although it was not always possible to identify the nest robber, it was often evident, in such cases, that predation rather than some other agency was responsible. In 19 instances it was plain that some predator, rather than anything else, had been the cause of nest failure, but it was not possible to be more specific.

Direct destruction of 28 nests was attributed to crows. The secondary depredation of nests from which cover had been removed by mowing, grazing, or some other factor, is not included in this figure. Such losses have been charged to the primary agent. Nests robbed by crows were placed in the following types of cover: slough, 9; pot hole, 1; fencerow, 5; railroad right of way, 4; roadside, 3; brush, 2; wasteland, lakeshore, woods and ditch banks, 1 each.

In other words, of all the nest failures over the three seasons of this investigation, only 8.2 per cent have been traced to crows. This figure does not support the almost universal condemnation of the crow as a robber of pheasant nests, and seems particularly suggestive when compared with the losses brought about by man.

To predatory mammals have been traced the destruction of 19 nests: ground squirrel (largely *Citellus franklini*, perhaps also *C. tridecemlineatus*) 6, one each in wasteground, gravel pit and brush, 3 in railroad right of ways; badger, one roadside nest; skunk, one nest in brush; coyote or dog (probably coyote), one slough edge nest; cat or fox, one roadside nest; unidentified small mammal, 8 nests, one each in fencerow, pasture, ditch bank, railroad, grass meadow, roadside, slough and alfalfa; unidentified large mammal (probably man or dog), one roadside nest. Losses attributed to mammalian predators constitute 5.6 per cent of all failures.

Few instances of adult mortality were traced to predators during the nesting season. Whether or not this fact has any relation to Errington's (1934) theory of population vulnerability, which indicates that adult bobwhites well within the carrying capacity of their winter environment may

enjoy a relative security from enemies, is not known. Three pheasants were killed on the nest, one by a cat or fox, one by a coyote or dog, and one by an unidentified predator. Evidence indicated that 4 others had been attacked while on the nest by unidentified predators, but that they might have escaped. How many nest failures were caused by the killing of the hen pheasant away from the nest could not be determined. One cock bird, found within 25 feet of two occupied nests, had been killed under conditions which pointed strongly toward mink as the predator.

In 12 instances, there were observed nest failures which seemed definitely attributable to the pheasants themselves, rather than to outside influences. Both Stoddard (1931, p. 24) and Yeatter (1934, p. 28), writing of bob-whites and Hungarian partridges, respectively, remarked upon the fact that nests were begun which were later abandoned because the site did not prove wholly satisfactory to the nest builders. Seven pheasant nests which were taken to represent such nests were found, six of which contained a single egg. The seventh nest, containing 22 eggs, was found in a fencerow location which offered no concealment whatever. This nest was deserted, presumably because the site was unsatisfactory. Four clutches appeared to represent "compound sets" or dump nests. In the case of one nest, which was incubated for a period in excess of thirty days, all of the eggs were infertile. These 12 nests comprise 2.7 per cent of the nests regularly under observation, or 3.5 per cent of those which failed to hatch.

In the discussion of nest drainage it was shown that pheasant nests were not infrequently poorly placed in this regard. A number of nests hatched, during the dry weather, in locations which did not offer adequate drainage. Twenty, however, were destroyed by flooding following rains. Fifteen of these, 14 in slough cover and one in a pot hole, were submerged by a rise of water level, while 5, of which 3 were in roadside ditches, one in a fencerow and one along a railroad, were destroyed by a heavy flow of accumulated surface run-off, in the channel of which the nests were placed. Flooding accounted for the failure of 4.5 per cent of the nests under observation, or 5.8 per cent of those which did not hatch. It might be added that the locations of the 51 nests at Jefferson Slough, not considered in the discussion above, were flooded by heavy rains shortly after the fire.

Three nests, placed upon absolutely bare plowed ground, were destroyed by unknown predators, but it would seem that the vulnerability of the nest site, rather than the particular genius of the animal which removed the eggs, was the essential cause of failure.

Cause of desertion or destruction could not be diagnosed for 62 nests, comprising 13.9 per cent of all nests studied, or 18.1 per cent of those which did not hatch. Some—perhaps a large proportion—almost undoubtedly represent nests which were begun and abandoned in favor of more attractive sites. Twenty-two were found so long after their destruction that an evaluation of primary causes was out of the question. Predation unquestionably accounted for some of these latter nests. In the case of 3, the eggs had remained untouched after having been deserted weeks before. In several instances, eggs disappeared without trace from nests which were visited regularly. In this connection, it was noted that a few eggs disappeared, during the course of incubation, from 8 nests which

hatched. Human pilfering was suspected in the removal of 2 clutches, but could not be proved. A few unexplained failures, undoubtedly, were nests of hens killed by predators, along highways, or by pot shooters. A possible source of further loss, but one which has not been explored during this study, is that from parasites and diseases. Some of the unexplained nest failures may well have arisen through such causes.

A summary of nest losses, showing the total number and percentage of all losses attributed to each cause of failure, is given in table 8. By the addition, at the foot of the table, of the nests which hatched and those for which the data are incomplete, the record of every nest found during this study is presented.

SUMMARY

Five hundred and three pheasant nests were found by direct search and through reports of local cooperators, both in undisturbed cover and after the removal of concealment by mowing and burning.

Nests were found to be placed on the ground, generally in a slight natural or scooped out depression but occasionally raised a few inches above the surface, and lined with whatever materials were at the spot. Construction of a roof did not appear to be a regular part of the nest building operations, but nests were often placed in such a manner as to take advantage of the partial or complete protection from above offered by nesting cover. On the other hand, nests completely exposed above were observed in 30.2 per cent of all cases. In the placing of nests, no particular direction appeared to be favored for exposure or nest openings, and no relation to the distance to trees or water was noted. Drainage of nests varied from excellent to poor, and averaged good. Variation from season to season was observed.

In the case of nests in large blocks of relatively homogeneous cover, a preference for the edge zone, rather than a uniform scattering, seemed to be shown. It is suggested that this edge zone may be proportional to the depth of the block rather than consisting of a strip, the same for any area, of absolute depth.

The average sizes of completed clutches were: 1933, 12.3 eggs, range 8 to 17; 1934, 10.1 eggs, range 4 to 20; 1935, 12.4 eggs, range 8 to 26. Average for the three seasons: 11.2, range 4 to 26 eggs. A decline in the number of eggs per clutch with the advance of the nesting season was observed. Nests in which more than one pheasant had laid eggs, and single eggs, not in nests, which had been laid carelessly or at random were encountered.

Fertility varied but little between the three seasons, and averaged 93.1 per cent. Viability showed greater variation: 1.5 per cent of embryos died in the shell in 1933; 12.2 per cent in 1934; and 14.0 per cent in 1935. Of the 65 clutches for which the data are complete, 58.4 per cent were made up entirely of fertile eggs, but only 36.9 per cent hatched every egg. Most clutches contained one to several chicks which died in the shell. As the season of 1934 was unusually dry, and that of 1935 wetter than the average for this region, these losses may not represent the normal condition. More data from normal years are needed before coming to definite conclusions.

Types and important constituents of nesting cover have been described, and relative success of nesting attempts in each type recorded.

The percentage of successful attempts was observed to be low in all cover types for which the data are considered sufficiently extensive.

The nesting season was found to extend from early April through early September, but the majority of clutches were begun in the period from late April through early June. While the nesting curve for 1935 has a single peak, occurring in the period from May 21 to 30, that of 1934 has two peaks, with a distinct drop between. In view of the lack of data from normal years, it can only be suggested that the 1934 trough was the result of the drought of that season.

On the basis of 445 nests under regular observation, 76.9 per cent were unsuccessful. Causes of failure were apportioned thus: man, 52.3 per cent of all failures; predators, 19.3 per cent; abandonment of unsatisfactory sites, dump nests and infertile clutches, 3.5 per cent; flooding, 5.8 per cent; nests totally lacking in cover, 0.9 per cent; unexplained, 18.1 per cent.

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EXPLANATION OF PLATES

PLATE I

- Fig. A. Nest wholly concealed from above by overtopping marsh cover. The cover has been parted to expose the nest.
- Fig. B. Nest partially concealed from above. The cover has been slightly parted to expose the nest.
- Fig. C. Nest not at all concealed from above. The cover has not been disturbed.
- Fig. D. Incubating pheasant on nest slightly concealed from above. The cover has not been disturbed.

PLATE I

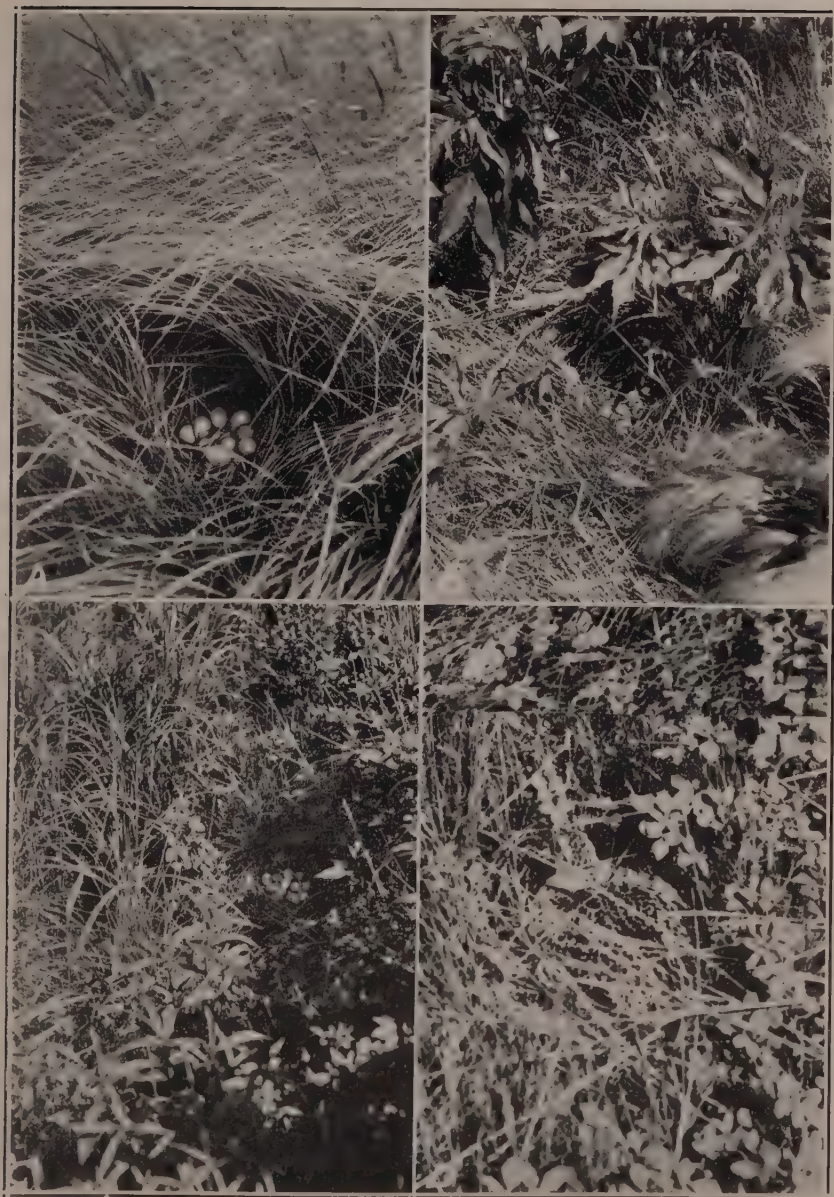


PLATE II

- Fig. A. Nest in slough cover. The cover has been parted to expose the nest.
- Fig. B. Nest in roadside cover. The cover has been parted to expose the nest.
- Fig. C. Nest in grass meadow cover. The cover has been parted to expose the nest.
- Fig. D. Nest in brush cover. The cover has been parted to expose the nest.

PLATE II



DETERMINATION OF ACETYLMETHYLCARBINOL IN FERMENTATION LIQUORS¹

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Dissimilation of carbohydrates and polyalcohols by many bacteria leads to the formation of acetylmethylcarbinol. Desmots (2) in 1904 detected this carbinol in cultures of *Bacillus subtilis*. Harden and Walpole (5) isolated and described it from cultures of *Aerobacter aerogenes*. Later acetylmethylcarbinol was recognized as the cause of the Voges-Proskauer reaction. Lemoigne (10, 12) found it present in cultures of species of *Proteus* and of *Bacillus anthracis*; Neuberg and Reinfurth (16), Kluyver, Donker and Visser't Hooft (7), and Elion (3) found it in yeast cultures; Lemoigne (9), Lafon (8) and Horowitz-Wlassowa and Rodionowa (6) showed it to be present in cultures of *Bacillus subtilis*; van Niel, Kluyver and Derx (20) detected it in cultures of *Streptococcus cremoris*, and with Michaelian, Farmer and Hammer (14) have shown the importance of the presence of acetylmethylcarbinol in butter cultures to flavor and aroma. Visser't Hooft and de Leeuw (21) found the carbinol in many samples of bread.

There has been no comprehensive report concerning the properties of this compound and its behavior during the course of the quantitative analysis of a typical fermentation liquor. Acetylmethylcarbinol used in this work was obtained from the Lucidol Corporation, Buffalo, N. Y.

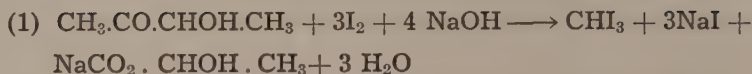
PURIFICATION AND PROPERTIES OF ACETYLMETHYLCARBINOL

The advertised purity of the commercial acetylmethylcarbinol used was "90% or better." It was a liquid when received, but slowly changed to an amorphous solid when held for a week to ten days at 0° C. The odor of diacetyl was evident. The presence of this compound is probably due to the slow oxidation of the carbinol.

An attempt was made to purify the commercial product by distillation under vacuum but decomposition took place. It was then mixed with cold, anhydrous ether, thoroughly stirred and filtered by vacuum. A snow-white, odorless, crystalline product was obtained which had no definite melting point. When held at a temperature of 85° C., it melted after 10 to 11 minutes. A small quantity of this liquid was placed at 0° C. and after 2 days crystals reappeared. The compound probably exists as a polymer in the solid state and a monomer in the liquid form or in aqueous solution.

The ether-washed acetylmethylcarbinol was tested for purity by iodine titration, using the Goodwin modification of Messenger's method (4). The reaction is represented by equation (1).

¹ Journal paper No. J293 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 65.



The carbinol solution was standardized by weighing the solid and making up to a definite volume. Based on calculations from successive titrations, one molecule of the carbinol required 6.07, 5.89, 5.97 and 6.04 atoms of iodine. Apparently, this product may be considered pure.

QUALITATIVE TESTS

Voges and Proskauer (22) described the production of an eosin-like coloration in glucose peptone cultures of certain organisms to which had been added a 10 per cent solution of potassium hydroxide. Harden found the reaction due to the presence of acetylmethylcarbinol and stated that the latter is oxidized by the addition of KOH and the resulting diacetyl reacts with proteins to give a red coloration. Various modifications of the original method have been suggested in order to hasten the appearance of the color. Levine, Weldin and Johnson (13) employed various oxidizing agents; Werkman (23) suggested the addition of FeCl_3 . O'Meara (17) tested for acetylmethylcarbinol by adding a small amount of creatine to 5cc. of the culture medium followed by 5 cc. of 40 per cent NaOH. After the addition of reagents, the tube is shaken vigorously. If the carbinol is present a red color appears in 1 to 2 minutes.

An aqueous solution of ether-washed acetylmethylcarbinol was diluted serially in order to determine the sensitivity of the O'Meara test. A definite color was present with a dilution of one part of the carbinol in 25,000 parts of water but not with one part in 50,000. Thus, the method has a great degree of sensitivity and the color develops quickly.

QUANTITATIVE METHODS

Lemoigne (11) described a specific reaction for the detection of acetylmethylcarbinol which consists in adding FeCl_3 solution to a portion of the culture and distilling the mixture. This process oxidizes the carbinol to diacetyl. The distillate is added to a mixture of ammonium hydroxide, hydroxylamine hydrochloride and nickelous chloride. The diacetyl is converted into dimethylglyoxime and the latter reacting with the nickel salt forms the red precipitate, nickel dimethylglyoxime.

Van Niel (19) found that although the Lemoigne reaction was very sensitive, it gave neither quantitative nor constant results. Van Niel's modification consists of adding an excess of 50 per cent FeCl_3 solution to an aliquot portion of the culture, slowly distilling, and collecting the distillate in a mixture, which for each 100 milligrams of acetylmethylcarbinol contains about 2 cc. of 20 per cent hydroxylamine hydrochloride, 3 to 5 cc. of 20 per cent sodium acetate and 1 to 2 cc. of 10 per cent nickelous chloride solution. When approximately three-fifths of the original liquid is distilled over, the flask containing the distillate mixture is closed and immersed for one hour in a water bath at 80°C . The precipitate is filtered, dried and weighed. The weight of acetylmethylcarbinol = $0.610 \times$ weight of precipitate. The author reported good quantitative results. It should be noted that van Niel substituted sodium acetate for the ammonium hydroxide which Lemoigne used.

In the determinations carried out in this laboratory, the van Niel modification of the Lemoigne method was followed. Twenty-five cc. of 50 per cent (hydrated) FeCl_3 solution were added to 50 cc. of the culture and about three-fourths of the mixture was distilled directly into a flask containing hydroxylamine hydrochloride, sodium acetate and nickelous chloride in the amounts suggested by van Niel. The tightly stoppered Erlenmeyer flask containing the distillate mixture was heated on a steam bath for at least an hour. After cooling, the precipitate was filtered through a weighed sintered-glass crucible, washed with hot water, dried for an hour at 110°C . and weighed.

In order to determine whether the van Niel method gives quantitative and constant results, various aliquot portions of a standard acetylmethylcarbinol solution were used with the procedure outlined (table 1).

TABLE 1. *Determination of acetylmethylcarbinol by the method of van Niel*

| Number | Solution used | Acetylmethylcarbinol used | Acetylmethylcarbinol found | Percentage recovery |
|---------|---------------|---------------------------|----------------------------|---------------------|
| 1 | c.c. 10 | gms. 0.0082 | gms. 0.0069 | 84.2 |
| 2 | 40 | 0.0328 | 0.0272 | 83.0 |
| 3 | 50 | 0.0410 | 0.0347 | 84.7 |
| 4 | 75 | 0.0615 | 0.0519 | 84.5 |
| 5 | 100 | 0.0820 | 0.0692 | 84.5 |
| 6 | 150 | 0.1230 | 0.1018 | 82.7 |
| 7 | 200 | 0.1640 | 0.1357 | 82.7 |
| Average | | | | 83.8 |

The values listed in table 1 indicate that the method of van Niel gives constant results and can be used conveniently, but that only approximately 84.0 per cent of the carbinol is thus determined. The method of calculation is represented in equation (2).

$$(2) \text{ Weight of Acetylmethylcarbinol} = \frac{\text{Wt. ppt.} \times 0.61}{0.84}$$

Efforts were made to improve the yield by (1) changing the concentration of FeCl_3 , (2) varying the fraction of the mixture distilled over, (3) using other oxidizing agents, (4) distilling under a partial vacuum, (5) distilling in an atmosphere of nitrogen, and various combinations of these methods. The yield was improved very slightly (85.5 and 86.2 per cent recovery) by distilling under a partial vacuum but conditions are more difficult to duplicate than when ordinary distillation is employed. Other attempts were unsuccessful.

THE RELATION OF ACETYLMETHYLCARBINOL TO THE DETERMINATION OF FERMENTATION PRODUCTS

The determination of acetylmethylcarbinol as outlined above is run on the fermentation liquor without preliminary treatment. The test is specific and can be used in the presence of other compounds. Aliquot portions of the liquor are used for the analysis of other fermentation products.

Acetylmethylcarbinol is fairly volatile and a considerable fraction is distilled over with the neutral solvents. The method used in this laboratory for determining the neutral solvents is described by Stahly, Osburn and Werkman (18). The alcohols are oxidized to acids by use of $K_2Cr_2O_7 + H_3PO_4$. One molecule of acetylmethylcarbinol is oxidized to two molecules of acetic acid by this procedure. The carbinol may be determined by the van Niel method on the neutral solvent fraction and deduction made for it in the total acetic acid found.

Fermentations which produce acetylmethylcarbinol generally also produce 2,3-butylene glycol. Brockmann and Werkman (1) published a method for the determination of the latter. In order to determine the effect of the acetylmethylcarbinol on this determination, a solution was made of a sample of the commercial product. The molarity of this solution was determined by the method of van Niel, making the correction outlined above. Thus, the molarity of the carbinol solution equals,

$$\frac{W \times 0.61}{0.84 \times 8.8},$$

in which W is the weight of the nickel dimethylglyoxime precipitate obtained from 100 ml. of the solution.

Aliquots of the carbinol solution were treated in the manner prescribed for the determination of 2,3-butylene glycol. The results given in column 3, table 2 indicate that any acetylmethylcarbinol in the fermentation liquor will react in the procedure for 2,3-butylene glycol and must therefore be determined separately and subtracted from the total given by the method of Brockmann and Werkman.

TABLE 2. *Determination of acetylmethylcarbinol*

| Aliquot Number | Method of van Niel (adjusted) | 2,3-butylene glycol method (Brockmann and Werkman) |
|----------------|-------------------------------|--|
| | gms. | gms. |
| 1 | 0.0510 | 0.0497 |
| 2 | 0.0600 | 0.0588 |
| 3 | 0.0245 | 0.0245 |
| 4 | 0.0495 | 0.0502 |
| 5 | 0.0734 | 0.0735 |

The values listed for the 2,3-butylene glycol method were calculated from the acetaldehyde formed on the assumption that one molecule of acetylmethylcarbinol when subjected to periodate oxidation yields but one molecule of acetaldehyde. These values correspond well with the adjusted values obtained by the method of van Niel. If one molecule of the

carbinol when oxidized with potassium periodate yielded two molecules of acetaldehyde, the values by the 2,3-butylene glycol method would be only one-half as great and therefore entirely out of line.

It is evident, therefore, that the molarity of 2,3-butylene glycol in a fermentation liquor is,

$$\frac{\frac{X - Y}{F} - M}{2},$$

in which

X = ml. N $\text{NH}_2\text{OH} \cdot \text{HCl}$ present in absorption tower.

Y = ml. N $\text{NH}_2\text{OH} \cdot \text{HCl}$ remaining unused in absorption tower.

F = ml. original fermentation liquor in the steam distillate used.

M = Molarity of acetylmethylcarbinol in the fermented liquor.

Brockmann and Werkman, in describing the periodate method of determining 2,3-butylene glycol, assumed that one molecule of the carbinol yielded 2 molecules of acetaldehyde. Their results were not adjusted to the purity of the carbinol which they used.

ACETYLMETHYLCARBINOL IN REDUCING SUGAR ANALYSES

Acetylmethylcarbinol reduces Fehling's solution precipitating cuprous oxide. When determinations of reducing sugar are made on fermentation liquors containing the carbinol, the latter must be determined separately. The amount found to be present is used to calculate the weight of cuprous oxide which it precipitates. This weight is subtracted from the total cuprous oxide to get the oxide precipitated by the sugar.

A solution of ether-washed commercial acetylmethylcarbinol was made by weighing out the crystals and making up the solution to a definite volume. Various aliquot portions of this solution were used with the Munson and Walker (15) method of sugar analysis. The solutions were heated at such a rate that boiling began in 4 minutes and was continued for 2 minutes. The cuprous oxide precipitate was filtered off, washed, dried and weighed. As indicated by the last column in table 3 the weight

TABLE 3. *Reduction of Fehling's solution by acetylmethylcarbinol*

| No. | Grams Cu_2O precipitated | Grams A.M.C. in sample | Ratio: |
|-----|---|---------------------------|---|
| | | | $\frac{\text{Grams } \text{Cu}_2\text{O}}{\text{Grams A. M. C.}}$ |
| 1 | 0.0245 | 0.0081 | 3.03 |
| 2 | 0.0520 | 0.0163 | 3.19 |
| 3 | 0.0603 | 0.0203 | 2.97 |
| 4 | 0.0725 | 0.0244 | 2.97 |
| 5 | 0.0850 | 0.0284 | 2.99 |
| 6 | 0.0979 | 0.0325 | 3.01 |
| 7 | 0.1066 | 0.0366 | 2.92 |

of cuprous oxide is a linear function of the weight of acetylmethylcarbinol. The weight of acetylmethylcarbinol is multiplied by 3 to obtain the weight of Cu_2O precipitated.

SUMMARY AND CONCLUSIONS

Commercial acetylmethylcarbinol can be purified by washing with anhydrous diethyl ether and filtering.

The Lemoigne-van Niel method of determining acetylmethylcarbinol gives constant but not quantitative results. By this method, approximately 84 per cent of acetylmethylcarbinol is determined.

One mole of acetylmethylcarbinol, when subjected to oxidation with $\text{K}_2\text{Cr}_2\text{O}_7 + \text{H}_3\text{PO}_4$, yields two moles of acetic acid. In the periodate oxidation for 2,3-butylene glycol, one mole of the carbinol forms one mole of acetaldehyde.

Acetylmethylcarbinol reduces Fehling's solution. In a reducing sugar determination, the Cu_2O precipitated by the carbinol must be subtracted from the total Cu_2O to obtain that due to the sugar.

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NOTES ON CUCURBITA MOSCHATA, DUCH.¹

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Pumpkins are commonly interplanted with corn and occupy a humble role in crop production, yet in the aggregate the annual pumpkin pack constitutes an important item of commerce in the Middlewest.

The cultivated varieties of cucurbits embrace three species—*Cucurbita maxima*, *C. pepo* and *C. moschata*; the latter species is the most variable. Duschene (5) described *C. moschata* as “tres difficile à circonscrire,” very difficult to delimit. Lamarck (9) questioned the validity of this species and classed it as a botanical variety of *C. pepo*.

Duchesne's description of the plant is not entirely clear as he refers to the fruit as being esteemed when eaten raw in certain countries, and the selection of the term *moschata*² on account of the musk-like odor leads one to question whether or not he was confused and perhaps had before him a specimen of a muskmelon rather than a pumpkin when completing his description of *C. moschata*. So far as we have been able to learn, in no country are pumpkins “eaten raw,” nor do they possess a pronounced musk-like odor. However, it should be noted that Duchesne's characterizations are accepted by Cogniaux (4) and that qualities such as aroma, flavor and edibility, though important to the horticulturist, are not taxonomic characters.

The species delineations are further complicated by the development and introduction of new varieties. Progress rests upon the securing of varieties better adapted to certain purposes, as canning or to particular regions. Such adaptations to use or environment involve modification which in turn requires a review of the species' taxonomic characters.

Castetter and Erwin (3) commented that certain inconstant characters of this species suggest the possibility of a hybrid origin. More recent genetic studies have revealed interesting data on the question of *C. moschata* being a hybrid species. A number of inbred lines of this species embracing a total of over 700 plants were developed at the Iowa Agricultural Experiment Station, and the taxonomic characters covering three generations show no reversion to other species; these results clearly refute the theory of a hybrid origin of this species.

The infertility of certain of the above hybrids (7) suggests that the crosses were made over species lines. The crosses between *C. moschata* and *C. maxima* resulted in 93 per cent of the fruits being parthenocarpic and those which produced fertile seed showed a marked tendency in the F₁ generation to produce abortive staminate flowers.

Crosses between *C. moschata* and *C. pepo* produced no fertile seed. In the reciprocal cross, however, fertile seed was maintained through the

¹ Journal Paper No. J288 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 298.

² Derived from the Latin *muscus*, the adjective form of the word which is applied to a number of plants possessing a musk-like odor.

third generation, indicating that the taxonomic relationship is closer between them than to the third species, *C. maxima*. Bailey likewise maintained a cross between *C. pepo* and *C. moschata* for three generations and noted that the pepo characters tended to become more and more dominant. These results clearly indicate that fertile crosses between *C. pepo* and *C. moschata* are possible. On the other hand, note the authors, "we are convinced that such crosses seldom if ever occur in nature." Varieties representing these two species are widely planted in the same locality in the corn-belt without evidence of crossing.

The fact that *C. moschata* has maintained its identity from pre-Columbian times to the present day in regions where there is abundant opportunity for crossing with *C. pepo*, rather emphasizes the fact that species lines here exist which have proven a barrier to inter-species crosses.

It is of interest also to find that the Seminole Indians of the Everglades of Florida grow pumpkins which they claim to be very old—"their fathers grew them always," is their story. The Seminole pumpkin is identified by Bailey (2) as *C. moschata*. Specimens collected by the writer closely resemble the Cushaw and Sweet Cheese varieties of the present day.

Recent archaeological studies also have given us added information on the possible origin and history of this species. Recoveries from the Cliff Dweller ruins and other historic regions of the Southwest have brought forth numerous well-preserved specimens of rinds, peduncles and seeds which have been identified by the writer as *C. moschata* (6). Some of these specimens, particularly those of Kidder and Guernsey (8) recovered from the Basket Makers, represent a culture antedating the Cliff Dwellers and are regarded by archaeologists as the most ancient peoples of whom we have any knowledge on the North American continent. "It seems quite possible," notes Kidder (8), "that the Basket Makers as we know them lived as long ago as 1500 or 2000 years before Christ."

The existence of specimens from pre-Columbian times, supported by the Seminole pumpkin which the Indians of this tribe claim is one of their ancient food plants, points rather definitely to the conclusion that *C. moschata* is an ancient American species.

ABERRANT CHARACTERS

PEDUNCLE

In the horticultural literature of cucurbits, pedicel characters are emphasized as one of the distinguishing features of this species. The furrowed stem with a swollen bell-shaped base (Pl. I, fig. A), as found in Sweet Cheese, has been frequently figured to illustrate the peduncle character of this species. Naudin (10) described *moschata* as having a "peduncle which broadens into five fingers at the point where it is inserted into the fruit." This type of fruit stalk, though typical of Sweet Cheese, a leading commercial variety, is not a constant character. In the White Cushaw, for example, the ridges of the fruit stalk terminate in a series of knob-like formations, and the bell-shaped base is entirely lacking (Pl. I, fig. B); while in other varieties of Cushaw the pedicel terminates in a bell-shaped base, but the stalk is clavate (Pl. I, fig. C) and often shows

scarcely a trace of furrowing; whereas, the Japanese Pie (Pl. I, fig. D) possesses a cylindrical pedicel, but the base is neither swollen nor flanged. It is interesting to note that the varieties possessing a pear-shaped fruit seem to trend toward a cylindrical pedicel.

LEAF CHARACTERS

In general the moschatas are characterized by silver spots at the intersection of the veins, as shown in Plate II, figure B, of variety Sweet Cheese, while in other varieties, as Golden Mammoth Cushaw, the silver marking may be entirely absent or only faint.

In most varieties the sinuses are indistinct or absent in comparison to the deeply sinused varieties of *C. pepo*. In some of the cushaws, however, the leaves are deeply sinused with a pronounced lobing, as shown in Plate II, figure B.

FLOWERS

In several varieties of moschata the sepals are linear; in others there is a slight broadening of the tip, while in others the foliaceous calyx is quite pronounced. See Plate III, figure A.

Long anthers characterize most varieties, though to this rule there are also notable exceptions, Plate III, figures C and D.

THE SEED

Seed characters afford an important means of identifying varieties belonging to *C. moschata*. So far as we have been able to observe, the fimbriated margin (fig. 1) of a deeper color than the body of the seed is a constant character in all of the cultivated varieties of this species offered in the North American seed trade. This is true also of the European sorts furnished this station by the Bureau of Plant Introduction, U. S. D. A., and in a number of varieties observed by the writer growing in Mexico.



Fig. 1. A thread-like margin of a darker shade characterizes the seed of *C. moschata*.

In two of the varieties, Japanese Pie and Green Striped Cushaw, the body of the seed is sculptured. An exception to the tawn colored margin is found in two Mexican varieties—Tamala and Xochimilco, in which the border is of a greenish cast. In these two varieties the seeds are linear to oblong, in contrast to obovate, typical of other varieties.

Russell (11) has made a valuable contribution to the literature of cultivated cucurbits by devising a key whereby the species identity of any variety may be determined by means of the external characters of the seed. The seed scar of *C. moschata* is described by

him as "normally truncate or rounded." However, as reported by Castetter and Erwin (3), the seed scar of this species varies widely and is not regarded by the author as a dependable character.

Is *C. moschata* a valid species? After eliminating the variable characters noted in the foregoing discussion, do there remain taxonomic limitations, upon which we may base a species? There are two important constant characters of *C. moschata*; the fimbriated or thread-like wavy margin of the seed and the tomentum of the vines and leaves. This type of seed is found in all of the varieties of *C. moschata* and in no other cultivated species of this genus, so far as we have observed. The tomentum is likewise a dependable character (Pl. II, fig. C). "The soft, hairy, grayish character of the plant and the absence of stiff setae sufficiently distinguish it," concludes Bailey (1).

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PLATE I.

Fig. A. A typical pedicel of *Curcubita moschata*.

Figs B, C, D. Aberrant forms. B and C. Varieties of White Cushaw showing variations in peduncle. D. Japanese Pie.

PLATE I.

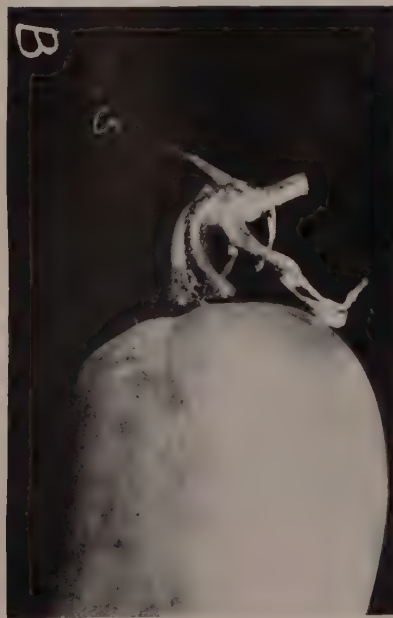
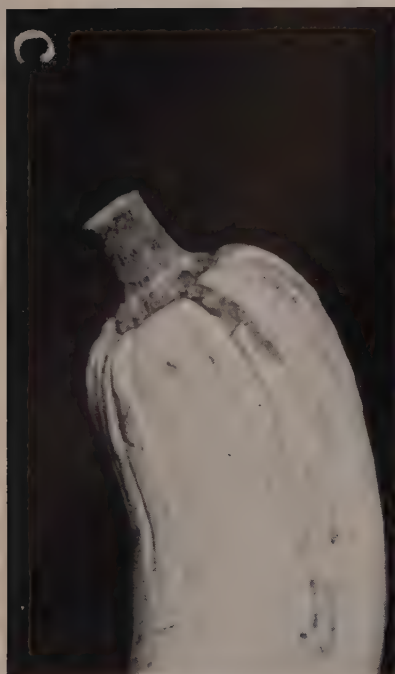


PLATE II.

Figs. A and B—The leaves of *C. moschata* vary in outline from entire to deeply sinused.

Fig. C.—A heavy coating of soft, silvery hairs and declinate tips characterize *C. moschata*.

PLATE II.



PLATE III.

FOLIACEOUS AND NON-FOLIACEOUS SEPALS OF *C. MOSCHATA*

Fig. A. Foliateous sepals.

Fig. 13. Non-foliateous sepals.

Figs. C and D—Long and short stamens.

PLATE III.



FOUR NEW THYSANOPTERA, WITH A PRELIMINARY LIST OF THE SPECIES OCCURRING IN IOWA¹

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One of the earliest systematic papers on Thysanoptera in America was the "Thripidae of Iowa," published by Miss Alice Beach (1895), in which thirteen species are recorded as occurring in Iowa. Since the appearance of her paper a number of entomologists have mentioned in widely scattered publications various forms collected in the state, but no other list has been published up to this time.

The present paper records eighty-five species from Iowa, four of which—*Frankliniella andropogoni*, *Eurythrips flavacinctus*, *Hoplothrips flavus*, and *Hoplothrips quercus*—are described below as new to science. All of the collections were made during the past four years by the junior author, many specimens being discovered, with the aid of a Berlese funnel, during the winter months in moss and dead leaves.

The list is by no means complete, as species previously unrecorded for the state are being found constantly. The paper is rather a list of those species so far identified.

The two new species of *Hoplothrips* are of special interest because on first observation they would not seem to belong to this genus. The almost complete fusion of the last two antennal segments, with only an incomplete suture separating the segments, is rarely found among the species of the genus. Priesner has recorded one, *H. ripicola* Priesner, in Europe. The eighth antennal segment may be small and closely joined, but the separation is usually clearly indicated.

DESCRIPTIONS OF NEW SPECIES

Frankliniella andropogoni, sp. nov. (Fig. 5)

Female holotype: Color, uniformly clear yellowish, including legs and wings; antennal segments one to three clear yellow, four shading to light brown, five brown, lighter at extreme base, six to eight dark brown. All spines clear yellow. Ocellar crescents deep orange.

Total body length, 1.05 mm.; head length, .143 mm., width .15 mm.; prothorax length .133 mm.; width .176 mm. Segments of antenna, length (width) II, 33 (26); III, 42 (20); IV, 40 (20); V, 36; VI, 50; VII, 10; VIII, 13 microns. Length of spines: interocellar 46, postocular 20; on prothorax, anterior marginal 26; on anterior angles 56, posterior angles, outer 60, inner 66; on ninth and tenth abdominal segments 166 microns.

Head somewhat angular in front as in *tenuicornis*, sides straight and parallel. Ocelli small, with distinct but small crescents. Base of third antennal segment simple, with only a slight swelling in outer half. Spines on

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² Entomologist, Redwood City, California.

fore wings; fore veins 16, hind vein 13. Comb on eighth abdominal segment wanting.

Type material: female holotype, nine female paratypes, taken on *Andropogon furcatus* Muhl., and nine female paratypes taken from bluegrass sod December 31, 1934, by Floyd Andre. Types in collections of both authors and Iowa State College. (Moulton No. 5355.)

Type locality: Waukon, Iowa.

This species may be separated from *genuina* Hood by its clear yellow color and yellow spines. *Genuina* has the prothorax mottled with brown and tergites of abdomen with irregular brown blotches, also with brown spines. The species may be separated from *spinusus* Moulton by the uniformly brown sixth antennal segment, in *spinusus* the sixth segment is clear yellow in basal third.

Eurythrips flavacinctus, sp. nov. (Figs. 3 and 6)

Female holotype: color head, abdominal segments one, two and six to nine also tube dark brown; thorax, all legs and abdominal segments three to five clear yellow; antennal segments one and two brown concolorous with head, three clear yellow, four to eight shading gradually from yellowish brown to brown.

Total body length 1.6 mm.; head length .26 mm., width .19 mm.; prothorax length .147 mm., width including coxae .32 mm.; tube length .19 mm., width at base .08 mm. Segments of antennae: length (width) II, 50 (34); III, 70 (33); IV, 63 (30); V, 63 (28); VI, 60 (23); VII, 50; VIII, 43 microns. Spines: postocellar 26 microns, postoculars 93, inner postoculars 53; on prothorax, anterior margin 56, anterior angles 53, midlateral 76, posterior angles, outer 80, inner 86; on ninth abdominal segment 200, at tip of tube 200 microns.

Head clearly constricted behind eyes, cheeks slightly arched, roughened, with one or two short spines on either side; eyes moderately large, with about six facets on the profile; ocelli small, placed far forward; postocular spines long and like other prominent body spines, with dilated tips; a second and shorter pair of postoculars inward from the others.

Prothorax with all normal spines well developed; fore legs somewhat enlarged, each fore tarsus armed with a small tooth. The holotype is without wings. One paratype has fully developed wings which are darkened with greyish brown in the second and fourth quarters comparing with the darkened areas on the body; each fore wing with six double fringe hairs.

Abdomen normal; tube two-thirds as long as head.

Male allotype; wingless; colored as in female; spines on ninth abdominal segment, outer 46, inner 133 microns; stronger tarsal tooth.

Type material: female holotype (wingless), male allotype (wingless), one female paratype with fully developed wings, thirteen female paratypes without wings, three male paratypes without wings, taken in moss in February and March, 1934, and in sod of *Andropogon furcatus* Muhl. in January, 1935, by Floyd Andre. Types in collections of both authors and Iowa State College. (Moulton No. 5357.)

Type locality: Ottumwa, Iowa.

This species is most closely related to *collaris* Hood, but is easily distinguished by the well developed spines on anterior margin of prothorax, the armed fore tarsi and the distinctly banded color.

Hoplothrips quercus, sp. nov. (Figs. 1, 2 and 4)

Female holotype: Color dark brown, including antenna, except only pedicle of third segment, and legs except all tarsi and extreme tips of all tibiae, which are yellowish. Thorax and abdomen with red pigment.

Total body length 1.78 mm.; head length .29 mm., width .26 mm.; prothorax length .235 mm., width, including coxae .396 mm.; tube length .16 mm., width at base .09 mm. Segments of antennae: length (width) II, 60 (36); III, 66 (36); IV, 60 (36); V, 60 (33); VI, 56 (33); VII, 80 (30); total length 426 microns. Distance between bases of antennae 20 microns. Length of spines: postoculars 90 microns; prothorax, on anterior margin 56, anterior angles 56, mid-laterals 66, outer pair on posterior angles 66, inner 70 microns; on ninth abdominal segment and tip of tube 166 microns.

Head slightly longer than wide, cheeks parallel, somewhat widened behind, without conspicuous spines or markings; eyes reduced, with about four facets on anterior margin, semi-oval in shape; ocelli small; postocular spines long and like other prominent body spines with dilated tips.

Prothorax with all normal spines developed; fore femora slightly enlarged; each fore tarsus armed with a short, sharp tooth; wings wanting.

Abdomen broad; tube .55 as long as head. Prominent spines on posterior angles of abdominal segments with dilated tips except those on segments seven, eight and ten, which are longer than the others and pointed.

Male almost identical to the female in shape and color.

Type material: female holotype, male allotype seven female and three male paratypes taken from under scales of bark of *Quercus alba* L. in April, 1934, September 7, 1935, and adults reared from nymphs found on September 15, 1935, by Floyd Andre. Types in collections of both authors and Iowa State College. (Moulton No. 5358.)

Type locality: Boone, Iowa.

This species is distinct from other species known from North America in that the seventh and eighth antennal segments are fused into one with only an incomplete cross suture to indicate their union. In this respect it is most closely related to the European species *H. ripicola* Priesner. It differs from this species, however, by the smaller eyes, longer spines and more uniformly brown coloring. It resembles closely *H. flavus*, another species described in this paper, but is easily distinguished by its color.

Hoplothrips flavus, sp. nov. (Figs. 7, 8 and 9)

Female holotype: Color clear orange yellow, including legs, first three antennal segments, most of four, half of five and base of six; fourth antennal segment shaded light brown in outer half, fifth darker, sixth and seventh mostly dark brown. Scattered reddish pigment in head and body.

Total body length 1.75 mm.; head length .29 mm., width .235 mm.; prothorax length .176 mm., width, including coxae .41 mm.; tube length .176 mm., width at base .102 mm.; width between antennal pits 16 microns. Antennal segments: length (width) II, 60 (36); III, 76 (40); IV, 73 (40); V, 66 (36); VI, 63 (33); VII, 83 (30); total length 485 microns. Length of spines: postoculars 93 microns; prothorax, on anterior margin 60, anterior angles 73, midlaterals 76, posterior angles, outer 80, inner 76; on ninth abdominal segment and tip of tube 180-200 microns.

Head somewhat longer than wide, flattened in front, cheeks parallel over posterior two-thirds. Eyes greatly reduced, with only two or three

facets on anterior margin; ocelli very small; postocular spines long and with dilated tips like other prominent body spines.

Prothorax .58 as long as head, with all normal spines developed; fore femora slightly enlarged; each fore tarsus armed with a small sharp tooth; wings wanting.

Abdomen broad; tube .6 as long as head. Prominent spines on posterior angles of abdominal segments with dilated tips except those on segments eight and ten, which are longer and pointed.

Male almost identical with the female in shape and color.

Type material: female holotype, male allotype, and one male paratype, taken from moss with Berlese funnel, February 11, 1934, by Floyd Andre. Types in collections of both authors and Iowa State College. (Moulton No. 5359.)

Type locality: Ottumwa, Iowa.

This species, like *quercus*, is distinct in having the seventh and eighth antennal segments fused into one, with only an incomplete suture to indicate their union. *Flavus* is easily distinguished from *quercus* by its bright orange-yellow color.

Suborder TEREBRANTIA Haliday, 1836.

Family AEOLOTHRIPIDAE Uzel, 1895.

Genus AEOLOTHRIPS Haliday, 1836.

Aeolothrips albicinctus Haliday.

Ames, hibernating in grass, January 2, 1935.

Aeolothrips bicolor Hinds.

Ames, on violet, May 15, 1933; Ottumwa, hibernating in moss, January 7, 1932.

Aeolothrips fasciatus (Linné).

Ames, on dandelions, April 15, 1932; New Sharon, hibernating in weeds, December 1, 1933.

Aeolothrips nasturtii Jones.

Davenport, on onions, May 7, 1932.

Family HETEROTHRIPIDAE Bagnall, 1912.

Genus HETEROTHRIPS Hood, 1908.

Heterothrips arisaemae Hood.

Ames, in flowers of Jack-in-the-Pulpit (*Arisaema triphyllum* (L.) Schott), May 3, 1934.

Family THRIPIDAE Uzel, 1895.

Genus HELIOTHRIPS Haliday, 1836.

Heliothrips haemorrhoidalis (Bouche).

Ames, from various greenhouse plants during December, January and February.

Genus *HERCOTHRIPS* Hood, 1927.

Hercothrips fasciapennis (Hinds).

Ames, sweeping in weeds, July, 1935.

Hercothrips femoralis (Reuter).

This species has been found breeding in greenhouses during the winter months at Ames, Oskaloosa and Ottumwa.

Genus *ECHINOTHRIPS* Moulton, 1911.

Echinothrips americanus Morgan.

Found throughout the summer months breeding on a legume (*Desmodium* sp.) at Boone.

Genus *CHIROTHRIPS* Haliday, 1836.

Chirothrips manicatus Haliday.

Ames, sweeping grass, June, 1934; Ottumwa, in moss, December, January, March.

Chirothrips obesus Hinds.

Ottumwa, hibernating in moss, December, 1932, 1933, and January, 1935.

Genus *LIMOTHRIPS* Haliday, 1836.

Limothrips cerealium Haliday.

Ottumwa, hibernating in leaves, December, 1933.

Limothrips denticornis Haliday.

Waukon, hibernating in *Andropogon furcatus* Muhl. sod. December, 1934.

Genus *APTINOTHRIPS* Haliday, 1836.

Aptinothrips rufus (Gmelin).

Waukon, hibernating in moss, January, 1934; Ottumwa, hibernating in grasses, December, 1932, 1933, 1934.

Genus *SERICOTHRIPS* Haliday, 1836.

Sericothrips apicalis Hood.

Ottumwa, in dead leaves, October, 1934.

Sericothrips beachae Hood.

Ottumwa, hibernating in moss, December, 1933, 1934.

Sericothrips cingulatus Hinds.

Ottumwa, hibernating in moss, December, 1932; Boone, sweeping grass, August 15, 1933.

Sericothrips interruptus Hood.

Ottumwa, hibernating in moss, December, 1932.

Sericothrips sambuci Hood.

McGregor, in dead leaves, November, 1933; Ottumwa, moss, October, 1934.

Sericothrips tiliae Hood.

Ottumwa, hibernating in dead leaves, January, 1935.

Sericothrips variabilis (Beach).

Ames, leaves of basswood, throughout months of May to October.

Genus DENDOTHRIPS Uzel, 1895.

Dendothrips ornatus (Jablonowski).

Found in large numbers on the leaves of cultivated lilac (*Syringa vulgaris* L.) at Ames during July, August and September, 1935. This European species was first recorded in North America by Moulton (1931). Specimens were sent to him from New Jersey, where this form was found doing considerable damage to California privet (*Legustrum ovalifolium* Hassk.).

Genus ANAPHOTHRIPS Uzel, 1895.

Anaphothrips obscurus (Müller).

Ames, corn in greenhouse, January, February, March; Ottumwa, hibernating in dead leaves, March 1, 1935; bluegrass, September 2, 1935.

Genus SCOLOTHRIPS Hinds, 1902.

Scolothrips 6-maculatus (Pergande).

Ames, leaves of basswood, June. 1935.

Genus FRANKLINIELLA Karny, 1910.

Frankliniella andrei Moulton.

McGregor, hibernating in moss, December, 1933.

Frankliniella andropogoni new species.

Waukon, in clumps of *Andropogon furcatus* Muhl., and bluegrass sod December 31, 1934.

Frankliniella cephalica (Crawford).

Ames, on strawberry plants, April 4, 1935.

Frankliniella fusca (Hinds).

Ames, on white clover, yellow sweet clover, June 15, 1935; dandelion, May 3, 1934; Ottumwa, hibernating in moss, January 7, 1932.

Frankliniella gilmorei Morgan.

Ames, on horsemint (*Monarda mollis* L.), June to September.

Frankliniella nervosa (Uzel).

Ames, on corn, June, 1934.

Frankliniella occidentalis (Pergande).

Ottumwa, hibernating in moss, December, January, February.

Frankliniella runneri (Morgan).

Ames, on milkweed (*Asclepias verticillata* L.), July 20, 1934.

Frankliniella tenuicornis (Uzel).

Ames, on corn, October 8, 1934; Ottumwa, on grasses, July 3, 1933.

Frankliniella tritici (Fitch).

Ames, on leaves of mullein (*Verbascum thapsus* L.), January 7, 1934; Ottumwa, on alfalfa, June 1, 1935.

Frankliniella varicorne Bagnall.

Ames, on violets, May 15, 1932; Ottumwa, hibernating in moss, January 15, 1932.

Frankliniella williamsi Hood.

Ottumwa, in flowers of Aster, June 9, 1935.

Genus *TAENIOTHIRPS* Amyot et Serville, 1843.

Taeniothrips simplex (Morison).

Ames, in blossoms of dandelions, May 7, 1935; gladiolus, summer 1934 and 1935; Des Moines, gladiolus 1934 and 1935. This species is well known as the gladiolus thrips and was described under the name of *Taeniothrips gladioli* M. & S.

Taeniothrips dianthi Priesner.

Moulton (1929) reports finding two specimens of this species in some material collected from *Dianthus* sp., July 4, 1927, by Mr. S. C. Jones at Shenandoah, Iowa.

Genus *PSEUDOTHIRPS* Hinds, 1902.

Pseudothrips inequalis (Beach).

Ames, on Aster, Sept. 8, 1935.

Genus *CTENOTHIRPS* Franklin, 1907.

Ctenothrips bridwelli Franklin.

Ames, on leaves of Jack-in-the-Pulpit (*Arisaema triphyllum* (L.) Schott), June, July, August.

Genus *THRIPS* Linné, 1766.

Thrips abdominalis Crawford.

Ames, on yarrow, October 12, 1934.

Thrips albopilosus Uzel.

Ames, on various greenhouse plants, December, January, February.

Thrips nigropilosus Uzel.

Ames, on chrysanthemum, November 12, 1934; greenhouse, on pansy, January 7, 1935.

Thrips tabaci Lindeman.

Ames, on onions, corn, sunflowers, during the summer months; hibernating in mullein.

Thrips treherni Priesner.

Ames, on *Lactuca pulchella* (Pursh) D. C., June 12, 1935; Waukon, on dandelion, May 1, 1934.

Genus PLESIOTHRIPS Hood, 1915.

Plesiothrips perplexus (Beach).

Ames, under leaf sheaths of cattails (*Typha latifolia* L.), December, February, March, 1933, 1934.

Family MEROTHRIPIDAE Hood, 1914.

Genus MEROTHRIPS Hood, 1912.

Merothrips morgani Hood.

Decorah, under bark of river birch (*Betula nigra* L.), December, 1933.

Suborder TUBULIFERA Haliday, 1836.

Family PHLOETHRIPIDAE Uzel, 1895.

Genus CRYPTOTHRIPS Uzel, 1895.

Cryptothrips rectangularis Hood.

New Sharon, on bark and under bark scales of peach tree, April to June, 1934.

Genus CEPHALOTHRIPS Uzel, 1895.

Cephalothrips elegans Moulton.

Ames, from clumps of *Andropogon furcatus* Muhl., January, February and March; Ottumwa, hibernating in moss, December, 1933 and 1934.

Cephalothrips errans Moulton.

Ames, under bark scales of apple and oak, January, 1935.

Genus HOPLOTHRIPS Amyot et Serville, 1843.

Hoplothrips americanus (Hood).

Ottumwa, hibernating in moss, January, 1932.

Hoplothrips angusticeps (Hood).

Ottumwa, hibernating in moss, December, 1934.

Hoplothrips flavicauda (Morgan).

Jewell, under willow bark, April, 1932.

Hoplothrips flavus new species.

Ottumwa, hibernating in moss, February 11, 1934.

Hoplothrips pergandei (Hood).

Ottumwa, hibernating in moss, January, 1935.

Hoplothrips quercus new species.

Boone and Ames, under bark scales of white oak (*Quercus alba* L.), April, 1934; September 7, 1935; nymphs found in September reared to adults.

Hoplothrips smithi (Hood).

Ames, hibernating in moss, January, 1935.

Genus *EURYTHRIPS* Hinds, 1902.

Eurythrips flavacinctus new species.

Ottumwa, hibernating in moss, February and March, 1934; Waukon, hibernating in *Andropogon furcatus* Muhl., December, 1934

Eurythrips osborni Hinds.

Ottumwa, hibernating in moss, December 7, 1933.

Eurythrips tarsalis Hood.

Ottumwa, hibernating in moss, January, 1935.

Genus *LIOTHRIPS* Uzel, 1895.

Liothrips caryae (Fitch.)

Ames and Boone, on leaves of hickory, June to September, 1933.

Liothrips citricornis (Hood).

Ames, on hickory, May, 1934; Ottumwa, hibernating in moss, January, 1934.

Liothrips leucognis Hood.

Ames, under bark scales of white oak (*Quercus alba* L.), every month of the year.

Liothrips ocellatus Hood.

Ottumwa, hibernating in moss, January, 1932, February 1, 1933, February 17, 1935.

Liothrips sambuci Hood.

Boone, hibernating in moss, January, 1933.

Genus *RHYNCHOTHRIPS* Hood, 1912.

Rhynchothrips pruni Hood.

McGregor, under bark scales of wild cherry (*Prunus virginiana* L.), December, 1933, January, 1934.

Genus *LISSOTHRIPS* Hood, 1908.

Lissothrips muscorum Hood.

Ames, in moss, June, 1933; Ottumwa, in moss, January, 1934.

Genus *NEOTHRIPS* Hood, 1908.

Neothrips corticis Hood.

McGregor, under bark scales of white oak and apple, February 16, 1934.

Genus *ALLOTHRIPS* Hood, 1908.

Allothrips magacephalus Hood.

McGregor, under bark scales of white oak, February 19, 1934.

Genus *HAPLOTHRIPS* Amyot et Serville, 1843.*Haplothrips aculeatus* (Fabr.).

Ames, on mullein, January, 1932.

Haplothrips faurei Hood.

Ames, gall of grape *Phylloxera*, August 30, 1934.

Haplothrips graminis Hood.

Ames, under bark of white oak (*Quercus alba* L.), April, 1934.

Haplothrips leucanthemi (Schrank).

Ames, in clover blossoms, August 15, 1932.

Genus *BAGNALLIELLA* Karny, 1920.*Bagnalliella yuccae* (Hinds).

Ames, Ottumwa, McGregor, behind leaves of yucca (*Yucca filamentosa* L.), all months of the year.

Genus *LEPTOTHRIPS* Hood, 1909.*Leptothrips mali* (Fitch).

Ames, on leaves of basswood (*Tilia americana* L.), May 17, 1933; Ottumwa, hibernating in moss, January 7, 1935.

Genus *NEOHEEGERIA* Schmutz, 1909.*Neoheegeria verbasci* (Osborn).

Ames, on mullein leaves (*Verbascum thapsus* L.), throughout the year; McGregor, under apple bark, January 6, 1934.

Genus *GLYPTOTHRIPS* Hood, 1912.*Glyptothrips flavescens* Hood.

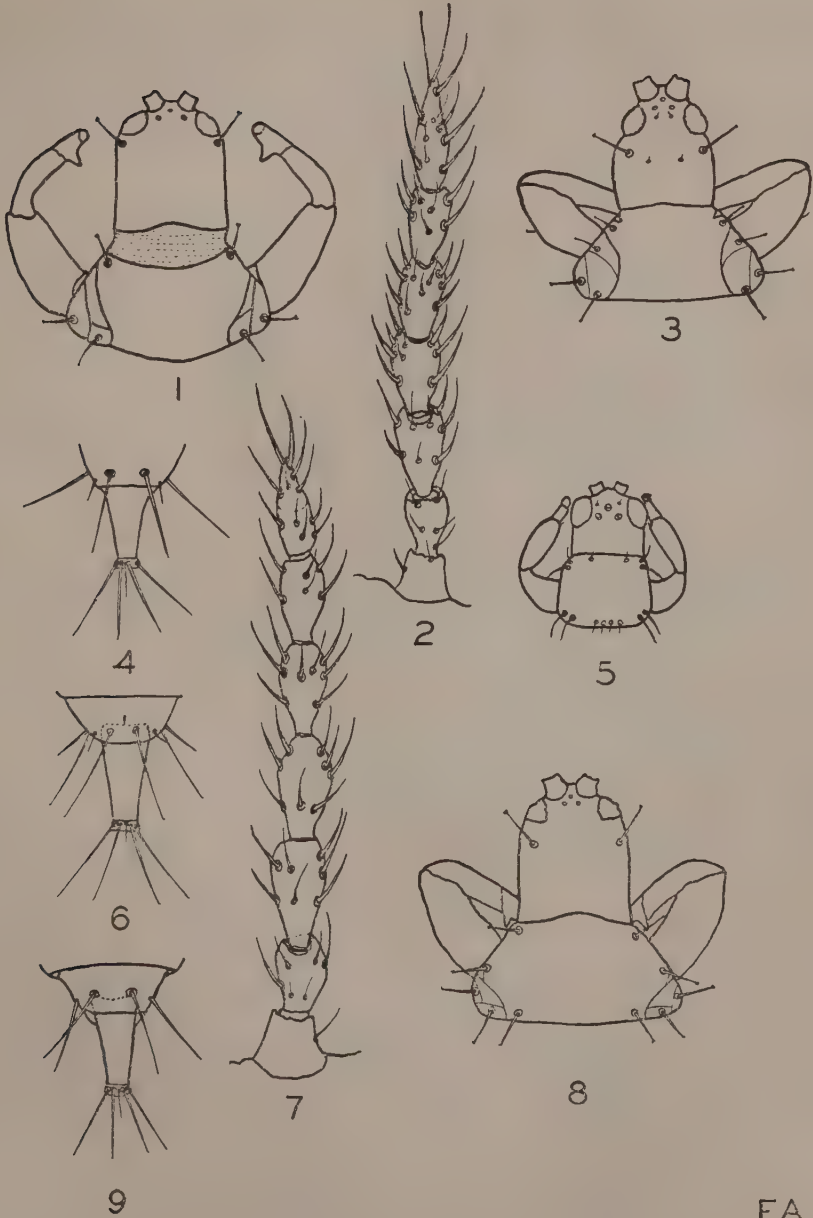
Ottumwa, hibernating in moss, January 2, 1932.

Genus *PHLOEOTHRIPS* Haliday, 1836.*Phloeothrips* (*Hoplandrothrips*) *xanthopus* Hood.

New Sharon, in moss, February 12, 1932.

DESCRIPTION OF FIGURES

- Fig. 1. *Hoplothrips quercus*, new species, head and prothorax of female.
Fig. 2. *Hoplothrips quercus*, right antenna of female.
Fig. 3. *Eurythrips flavacinctus*, new species, head and prothorax of female.
Fig. 4. *Hoplothrips quercus*, tip of abdomen of female, dorsal view.
Fig. 5. *Frankliniella andropogoni*, new species, head and prothorax of female.
Fig. 6. *Eurythrips flavacinctus*, tip of abdomen of female, dorsal view.
Fig. 7. *Hoplothrips flavus*, new species, right antenna of male.
Fig. 8. *Hoplothrips flavus*, head and prothorax of male.
Fig. 9. *Hoplothrips flavus*, tip of abdomen of male.



Phloeothrips (Acanthothrips) nodicornis Reuter.

Ames, under willow bark, May 1, 1934; New Sharon, under bark scales of willow, November 1, 1934. Bagnall (1933) described as new *Acanthothrips americanus* from some Michigan specimens, saying that it differed from European examples of *nodicornis*. If those specimens we are calling *nodicornis* are later shown to be identical with those from Michigan Bagnall described as *americanus*, the Iowa examples will of course be known under the name of *americanus* Bagnall.

Genus *NEUROTHRIPS* Hood, 1924.*Neurothrips magnafemoralis* (Hinds).

Ames, under willow bark, May 1, 1934; under bark scales of apple, April 7, 1935.

Genus *OEDALEOTHRIPS* Hood, 1916.*Oedaleothrips andrei* Watson.

This species was described (Watson, 1933) from four females and four males taken from grassy material around a haystack at Boone, Iowa, March 1, 1933, and at Ames, Iowa, January 25, 1933.

Genus *BOLOTHRIPS* Priesner, 1926.*Bolothrips bicolor* (Heeger).

Ames, sweeping grass, June, 1934; McGregor, moss, September 6, 1935; Boone, sweeping grass, September, 1935.

Genus *ELAPHROTHRIPS* Buffa, 1909.*Elaphrothrips armatus* (Hood).

Ames, dead leaves, October, 1934.

Elaphrothrips flavipes (Hood).

McGregor, dead leaves, September, October, 1933.

Elaphrothrips tuberculatus (Hood).

Ames, moss, September, 1932; dead leaves, January 5, 1935.

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RELATIVE TOXICITY OF CERTAIN STOMACH POISONS TO PHYLLOPHAGA LANCEOLATA (SAY) (COLEOPTERA- SCARABAEIDAE)¹

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The relative toxicity of stomach poisons to adult June beetles has been but little investigated and lack of knowledge on this subject has hindered recommendations concerning the use of poisonous materials for control measures. The cage experiments of Travis and Decker (1932) constituted one of the first attempts to study the effect of arsenicals upon adult June beetles. In their experiments, however, the amount of poison consumed was unknown. This paper was to ascertain the exact dosages required to kill June beetles.

MATERIALS

Although *Phyllophaga lanceolata* (Say) has been recognized as a common crop pest in Southwestern United States for many years, not until the past summer (1934) was it discovered to be abundant in a limited area of southwestern Iowa. This species is peculiar in that it is diurnal in habit, whereas all but two or three other species are nocturnal. This habit proved to be helpful in making tests since feeding occurred during the day instead of at night.

The beetles which were to be used as tests were gathered from pasture fields and placed in a large screened cage where fresh dandelion leaves were provided for them to feed upon until they were used in the experiments. In order that the beetles would feed well on the poisoned foliage offered them, they were starved twelve hours previous to use. On account of the heavy death rate of males, even in the controls, only female beetles were used in the experiments.

The relative toxicity of three arsenical compounds and one metallic cyanide are presented in this paper. Incomplete toxicity records are also given for two other arsenicals and one fluosilicate. The compounds used, with their pertinent analytical data are: paris green (CuOA_2O_3), $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2$, CuO 38.84 per cent, As_2O_3 54.75 per cent, water soluble As_2O_3 3.00 per cent; cuprous cyanide CuCN 99.34 per cent; arsenious oxide As_2O_3 99.89 per cent; acid lead arsenate (PbHAsO_4 , PbO 64.50 per cent, As_2O_5 32.88 per cent, water soluble As_2O_5 0.22 per cent; calcium arsenate CaO 33.16 per cent, As_2O_5 31.23 per cent, water soluble As_2O_5

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0.29 per cent; sodium fluosilicate Na_2SiF_6 96.71 per cent; and zinc arsenite, commercial sample.

The insecticides were administered with slight modifications, by means of the "leaf sandwich" method method of Campbell and Filmer (1929). Fresh potato leaf sandwiches dusted with the compound to be tested were used to apply the desired dosage. Bean and dandelion leaves were tried, but these were so thin that the sandwiches did not maintain their shape.

METHODS

A well sharpened cork borer was used to cut discs from the potato leaves. The discs were cut and retained in a moist chamber until needed. An aqueous solution of gum arabic having a consistency like that of honey was employed as an adherent for the insecticide filler of the sandwich, and also to hold firmly together the two discs of the sandwich. The gum arabic allowed heavier applications of the poison and fastened together the two sides of the sandwich better than did the starch paste used by previous workers.

The toxic material was applied to the leaves as a dust. It was first ground in a mortar and passed through a 200-mesh screen before being applied to the leaves. This dust was then blown into a bell jar, the larger particles were allowed to settle out, and the jar was moved over a glass plate which held sandwich discs and a piece of tared graph paper, 2260 sq. mm. in area. The increase in weight of the paper after dusting was used to calculate the amount of poison falling upon the surface of the discs.

In order to prepare sandwiches, unpoisoned discs were coated on one side with a film of the paste and pressed with the gummed side down, over the poisoned surface of the dusted discs. The sandwiches were fastened in wire clips which were inserted in the side of a large cork. The treated food was offered successively to caged beetles until one was found that would eat it. As soon as feeding had started, the cork bearing the sandwich and beetle was placed gently on a table and the beetle allowed to eat until satisfied or until it had consumed an amount of poison sufficient for a toxicity test. After feeding, the beetles were placed in large glass vials with unpoisoned food, and held in the laboratory for future examination. The body weights of the beetles were obtained after feeding, since many individuals refused to feed for a long time after the disturbance caused by this handling.

The dosages consumed were calculated from the number of square millimeters of the sandwich eaten. This consumed area was measured by placing the sandwich over a piece of millimeter graph paper with a circle drawn on it equal in size to that of the original disc. Measurements were made immediately after the beetles had fed in order to overcome errors resulting from shrinkage of the leaves. If shrinkage did occur, the leaves were placed in a moist chamber and measured when they had regained their original diameter.

Observations were made at twelve-hour intervals for a 96-hour period to ascertain the survival time of the poisoned beetles. Individuals were considered dead that were unable to move their legs when gently pressed on the ventral side of the abdomen.

The fifteen control beetles used each day received the following treatment during the observation period of 96 hours; five received no

food, five received leaves with a coating of unpoisoned gum arabic, and five received fresh untreated leaves. The number of beetles that died in these controls was almost equally distributed in the three different treatments. Only 6.6 per cent of the beetles died in the four-day period.

RESULTS

Gum arabic paste produced no visible toxic or repellent effects. Little difference in survival time was noted for the various dosages. The survival time of beetles fed sandwiches poisoned with arsenious oxide, paris green, acid lead arsenate and cuprous cyanide, are presented graphically in figure 1, where the percentage of survivors is plotted against time. At the second observation, twenty-four hours after the beetles fed upon the poison, paris green, cuprous cyanide, acid lead arsenate appeared to be equally effective, killing from 15 to 17 per cent of the insects during this interval. At the end of 48 hours, however, most of the beetles in the experiments had died; in only two cases did any beetles survive 96 hours; these had received arsenious oxide. Arsenious oxide is apparently much slower in lethal action upon this insect than any of the other three insecticides tested. After ingesting arsenious oxide, 50 per cent of the beetles survived for about 20 hours, whereas in the case of acid lead arsenate the 50 per cent survival point was reached in about 13 hours. The 50 per cent point was passed long before the first observation time (12 hours) for beetles fed paris green and cuprous cyanide.

From 15 to 20 minutes after having fed upon the poisoned sandwiches, many of the beetles regurgitated copiously. Undoubtedly much poison was thrown off in this manner, but the fact that only one beetle survived which had regurgitated indicates a retention of a lethal dose of the administered compounds. This one exception occurred in the paris green tests after 0.02 mg./gm. of this arsenical had been consumed. In the various tests, regurgitation occurred in the following percentages of beetles before death occurred: calcium arsenate 100 per cent, paris green 100 per cent, arsenious oxide 92 per cent, acid lead arsenate 89 per cent, sodium fluosilicate 75 per cent, copper cyanide 70 per cent, and zinc arsenite 64 per cent.

The foregoing data are shown in more detail in table 1. It is interesting to note that regurgitation occurred in a greater number of cases in beetles that were fed arsenicals, with the exception of those given zinc arsenite. None of the *P. implicita* (Horn) used in another test were seen to regurgitate even though several hundred were fed calcium arsenate.

PARIS GREEN

The experiments with paris green show this arsenical to be the most toxic of any tested to *P. lanceolata* (table 2). There was no significant difference between the mean dosages of the beetles that died and those that recovered in the intermediate zone. In view of this, the figure 0.03 mg./gm. can be considered to be the median lethal dose for this insect. Richardson and Haas (1931) found the M.L.D. for paris green to be less than 0.10 mg./gm. for *Leptinotarsa decemlineata* (Say) and the same authors (1932) found the M.L.D. for *Melanoplus femur-rubrum* (De G.) to be 0.19 mg./gm. The figure for *P. lanceolata* is then about one-sixth as large as the M.L.D. for *M. femur-rubrum*, and probably less than that obtained for *Leptinotarsa* larvae.

TABLE 1. Percentages of beetles that regurgitated after having eaten poison (*Phyllophata lanceolata* 1934)

| Insecticide | Arsenious oxide | Paris green | Zinc arsenite | Acid lead arsenate | Calcium arsenate | Cuprous cyanide | Sodium fluosilicate |
|---|-----------------|-------------|---------------|--------------------|------------------|-----------------|---------------------|
| Total No. insects | 49 | 57 | 12 | 173 | 70 | 104 | 12 |
| No. insects dead | 25 | 25 | 11 | 108 | 5 | 71 | 8 |
| Percentage that regurgitated before death | 92 | 100 | 64 | 89 | 100 | 70 | 75 |
| No. insects recovered | 24 | 32 | 1 | 65 | 65 | 33 | 4 |
| Percentage regurgitating that recovered | 0 | 4 | 0 | 0 | 0 | 0 | 0 |

CUPROUS CYANIDE

The estimated M.L.D. obtained for cuprous cyanide was 0.04 mg./gm. or about the same as that obtained for paris green. This was the same figure obtained by Campbell and Filmer (1929) for the silkworm. Richardson and Thurber (1933) obtained an M.L.D. of 0.11 mg./gm. for *Melanoplus differentialis* (Thomas), a figure almost four times the value

TABLE 2. A summary of the toxicity data for *Phyllophaga lanceolata* Say (1934)

| Zone | Poisons | | | |
|------------------------------|-------------|-----------------|-----------------|--------------------|
| | Paris green | Cuprous cyanide | Arsenious oxide | Acid lead arsenate |
| Lethal | | | | |
| Dosage range* | 0.27-0.07 | 0.47-0.08 | 0.24-0.15 | 1.59-0.24 |
| Mean dosage* | 0.11 | 0.16 | 0.13 | 0.45 |
| Number of insects | 11 | 37 | 5 | 61 |
| Range in weight (gms.) | 0.24-0.46 | 0.23-0.51 | 0.23-0.46 | 0.26-0.53 |
| Mean weight (gms.) | 0.38 | 0.35 | 0.36 | 0.42 |
| Intermediate | | | | |
| Dosage range* | 0.06-0.02 | 0.07-0.01 | 0.14-0.03 | 0.23-0.06 |
| Mean dosage that killed* | 0.034 | 0.043 | 0.066 | 0.14 |
| Mean recovery dosage* | 0.033 | 0.036 | 0.063 | 0.11 |
| Median lethal dose (M.L.D.)* | 0.03 | 0.04 | 0.06 | 0.12 |
| Number of insects | | | | |
| Died | 14 | 34 | 20 | 47 |
| Recovered | 13 | 28 | 17 | 36 |
| Range in weight (gms.) | 0.26-0.49 | 0.23-0.50 | 0.21-0.50 | 0.26-0.59 |
| Mean weight (gms.) | 0.37 | 0.36 | 0.36 | 0.41 |
| Sublethal | | | | |
| Dosage* | 0.02-0.007 | 0.008-0.005 | 0.03-0.01 | 0.06-0.009 |
| Mean dosage* | 0.01 | 0.06 | 0.02 | 0.03 |
| Number of insects | 19 | 5 | 7 | 29 |
| Range in weight (gms.) | 0.24-0.54 | 0.39-0.56 | 0.19-0.49 | 0.26-0.51 |
| Mean weight (gms.) | 0.38 | 0.43 | 0.37 | 0.42 |

* Dosages are given in milligrams of poison per gram body of weight of the insect.

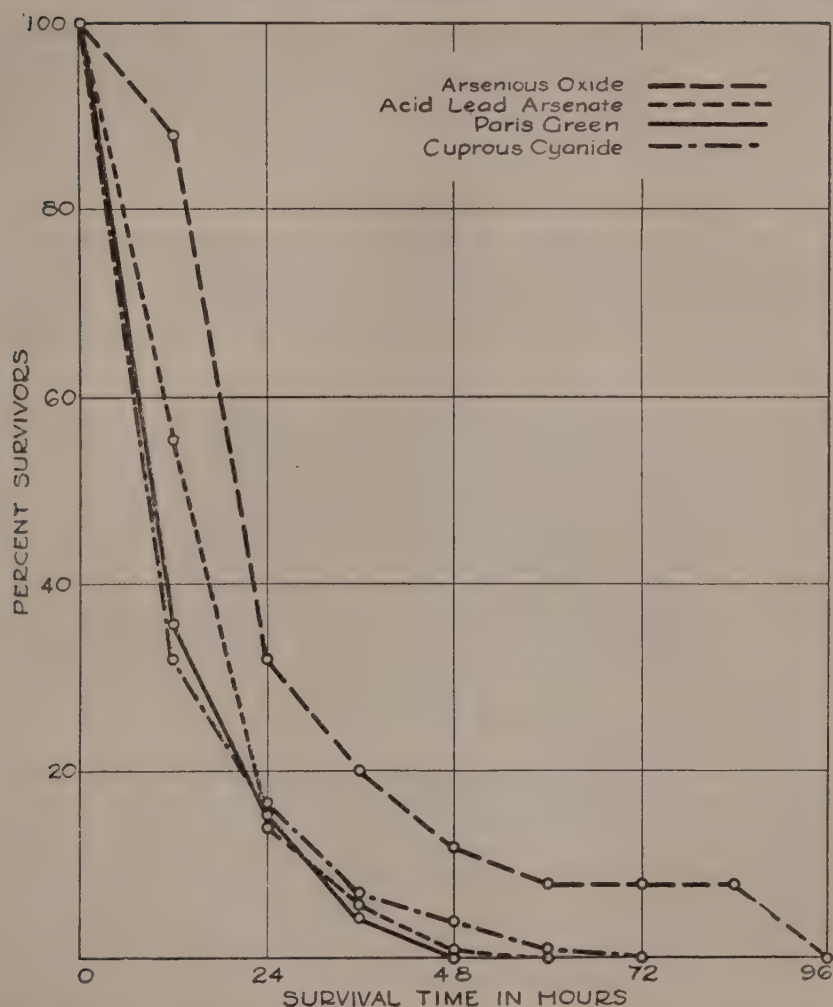


Fig. 1. Survival curve showing effectiveness of various substances fed to *Phyllophaga lanceolata* (Say). Dosage range, mg./gm. of body weight: arsenious oxide, 0.24-0.03; paris green, 0.27-0.02; cuprous cyanide, 0.47-0.12; acid lead arsenate, 1.59-0.06.

obtained for *P. lanceolata*. A comparison of the means showed no significant difference between them. A statistical comparison of the dosages within the intermediate zones of both cuprous cyanide and paris green indicates doubtful significance, so that as great a variation in the M.L.D.'s could possibly be expected in two groups of these insects which were fed the same poison.

ARSENIUS OXIDE

Arsenious oxide gave an M.L.D. of 0.06 mg./gm., which is two times as large as the figure obtained for paris green (table 2). The M.L.D. of

Richardson and Haas (1933) of 0.11mg./gm. for *M. differentialis* (Thomas) is nearly two times as great as the figure obtained for this insect. Campbell (1932) reported an M.L.D. of about 0.02 mg./gm. for the silkworm, a figure which is only one-third that obtained for *P. lanceolata*. A comparison of the mean dosages in the intermediate zone shows that they are not significantly different³.

ACID LEAD ARSENATE

The M.L.D. of acid lead arsenate of 0.12 mg./gm. is very near the figure of Campbell and Filmer (1929) of 0.09 mg./gm. for the fourth instar silkworm. A statistical comparison of the means, 0.14 mg./gm. and 0.11 mg./gm. shows a slight significance between them. The average of these means is, however, the best figure for the M.L.D. that can be obtained from these data.

CALCIUM ARSENATE

An attempt to secure an M.L.D. for calcium arsenate proved unsuccessful. Dosages of from 0.06 to 1.15 mg./gm., with a mean dosage of 0.39 mg./gm., in an evenly distributed series were administered to 70 beetles. Five of these died after consuming 1.15, 0.79, 0.50, 0.19, and 0.18 mg./gm., respectively, of the poison. Only five individuals regurgitated, and these were the five that died. Similar results were obtained when calcium arsenate from this same source was used in toxicity experiments on *P. implicata* (Horn). A few of the beetles died, but results were very erratic. A different brand of calcium arsenate gave fair results (Travis and Decker, 1933) when used in cage tests on *P. hirticula* (Knoch) and *P. rugosa* (Mels.). The poor showing made by the calcium arsenate used in the M.L.D. tests may indicate that the sample used was less toxic than that used in the cage tests.

Zinc arsenite and sodium fluosilicate were tried, but time did not permit completion of the tests before the beetles were gone; only twelve individuals were used in each test. In the case of zinc arsenite all of the beetles died except one. This one had consumed 0.08 mg./gm. of the compound. Dosages for those that died were 0.38, 0.28, 0.18, 0.13, 0.13, 0.12, 0.12, 0.09, 0.06 and 0.04 mg./gm., with a mean dosage of 0.15 mg./gm. Four of the twelve beetles lived after eating 0.45, 0.12, 0.11, 0.09 mg./gm., respectively, of sodium fluosilicate. The eight that died had eaten 1.29, 1.07, 0.99, 0.94, 0.75, 0.63, 0.13, 0.07 mg./gm. with a mean of 0.73 mg./gm.

DISCUSSION

Lead arsenate appeared to stimulate oviposition. Only beetles that were fed this poison laid eggs in the containers in which they were retained for observation. Of the 108 beetles that died, 61 laid a total of 136 eggs with a range of 1 to 5 and an average of 2.2 per beetle. Of the 65 that survived, 7 laid a total of 11 eggs with a range of from 1 to 4 and an average of 1.6 per beetle. These eggs were all fertile, except those that were covered with regurgitated materials.

Male beetles of this species were not used in the M.L.D. tests. As pointed out in the cage tests of Travis and Decker (1933) male *P. hirticula*

³ The data were all analysed with Fisher's test of significance for the means of small samples.

have a high death rate earlier in the season and are much more susceptible to insecticides than the females. This is also true for the male *P. lanceolata*; in fact, they proved to be affected a great deal more by hot weather and handling than the males of *P. hirticula*.

The *P. lanceolata* were not discovered in Iowa until rather late in the flight season, and at that time the weather was so extremely hot that the flight period was greatly shortened. Temperatures were so high in the laboratory at the time the experiments were in progress, that the beetles were kept alive only by placing them in a cool room.

It is of course axiomatic that the control of female beetles in the field is more important than the control of males, if females are destroyed previous to oviposition.

SUMMARY

Adult female June beetles, *Phyllophaga lanceolata* (Say), were administered various dosages of paris green, cuprous cyanide, arsenious oxide, acid lead arsenate, calcium arsenate, zinc arsenite, and sodium fluosilicate. Males were not used in these tests. The estimated median lethal dose for paris green was 0.03 mg./gm. of body weight; cuprous cyanide 0.04 mg./gm., arsenious oxide 0.06 mg./gm. and acid lead arsenate 0.12 mg./gm.; calcium arsenate from this sample evidently produced very little toxic effect; sodium fluosilicate seems to be of low toxicity; the zinc arsenite apparently has a relatively high toxic value for this insect. No M.L.D. was obtained for the three latter compounds. Beetles died even though they regurgitated freely after feeding upon the poison. Acid lead arsenate seemed to stimulate oviposition.

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THE TOXICITY OF CERTAIN STOMACH POISONS TO THE JUNE BEETLE, *PHYLLOPHAGA IMPLICITA* (HORN)¹

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Adults of the common white grub, or June beetles, have been responsible for considerable defoliation damage to both shade and forest trees in Iowa during the past 20 years. In numerous cases reported, entire woodlots have been practically denuded; and, in a few instances, most of the trees on certain golf courses were defoliated.

Numerous requests have been received at the Iowa Experiment Station for a method of controlling the beetles during their destructive period. Naturally enough, by poisoning the adults one would consequently reduce the population of grubs that would ordinarily be present the ensuing year. Previous to the present study virtually nothing was recorded in entomological literature concerning specific amounts of poison fatal to June beetles of nocturnal habit. Travis and Decker (1933) have reported successful kills by dusting a mixture of 40 per cent calcium arsenate and 60 per cent bentonite on leaves of oak under cage conditions, but they did not attempt to determine the specific amounts of poison fatal to the beetles. Travis (1936) worked with adults of *Phyllophaga lanceolata* (Say), a diurnal form, and determined four median lethal doses.

Before one can recommend poisons to be dusted on the foliage of trees and shrubs which may serve as preferred hosts for the feeding activities of imago *Phyllophaga*, a knowledge of the relative toxicity of the different stomach poisons is of paramount importance. As the present spring and summer (1935) was the period for Brood A results to be present, certain species could be obtained in abundance. These studies were therefore worked out under laboratory and field conditions, but only the laboratory phase of the problem will be dealt with in this paper.

MATERIALS

The species of June beetle selected for this study was *Phyllophaga implicita* (Horn). This particular form is nocturnal in habit. During the day it secretes itself in sod, beneath the surface of cultivated ground, under boards, logs, dead leaves, and in other similar situations; and with the approach of darkness it comes forth from its hiding place to seek its food plants. In the vicinity of Ames, as in most other sections of the state during 1935, it was by far the most abundant of the various species of June beetles, and could be collected in large numbers from willow and elm in almost any locality.

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² The writers wish to express their appreciation to Dr. C. J. Drake, who made the completion of this work possible, and to Dr. C. H. Richardson for his valuable suggestions.

The adult insects used in the experiment were collected by placing a large canvas under a branch of a medium-sized willow or elm where the beetles had congregated and then shaking the feeding beetles from the particular limb. A large number of specimens could be collected in this manner in a very few minutes. Usually the collections were made at about 10 p. m., or shortly after the beetles had settled on the foliage and had begun feeding. Immediately after the collection was made the beetles were taken to the screened insectary and confined in insect cages, each of which had a two-inch layer of soil in the bottom. Each evening they were fed on fresh willow leaves. To insure a fresh supply of beetles, collections were made every fourth day and the beetles on hand at the end of the four-day period were discarded.

Eight different poisons were used in the experiments: (1) calcium arsenate, (2) rotenone, (3) a commercial manganese arsenate, (4) acid lead arsenate, (5) paris green, (6) sodium fluoride, (7) cuprous cyanide, and (8) sodium fluosilicate. An attempt was made to determine the M. L. D. for each of these compounds. However, for reasons which will be discussed later, this was not practical in the case of the first three poisons. An M. L. D. was determined for each of the latter five compounds, however.

The insecticides used contained the following proportions of the various materials: (1) calcium arsenate (CaO 33.16 per cent, As_2O_5 31.23 per cent, water soluble As_2O_5 0.29 per cent), (2) rotenone (95 per cent rotenone), (3) manganese arsenate (As_2O_5 40 per cent, metallic arsenic 26 per cent, active manganese 68 per cent, inactive 32 per cent), (4) acid lead arsenate (PbO 64.50 per cent, As_2O_5 32.80 per cent, water sol. As_2O_5 0.5 per cent), (5) paris green (CuO 38.84 per cent; As_2O_3 54.75 per cent, water soluble As_2O_3 3.00 per cent), (6) sodium fluoride (99 per cent NaF), (7) cuprous cyanide (99.34 per cent CuCN) and (8) sodium fluosilicate (Na_2SiF_6 96.71 per cent).

METHOD

Disks 18 mm. in diameter were cut from freshly gathered elm leaves by means of a cork borer and then kept in a moist chamber until ready for use. The method of Campbell and Filmer (1929) was employed to obtain a dose of poison on thirty leaf disks. A square of millimeter cross-section paper, having an area of 2500 mm., was placed on the glass plate with the leaf disks. A dust cloud of the poison to be used was blown into a bell jar and allowed to settle on the leaves as well as on the squared-millimeter paper. Before the paper was dusted it was weighed on a chainomatic balance, and after the dusting was completed, weighed again. Thus by taking the difference between these two weights the weight of poison on 2500 mm.² of paper could be obtained, and from this was calculated the amount of poison on 1 mm.² of leaf.

After the dusting had been completed the leaf disks were removed to a clean paper and the sandwiches finished by placing on each poisoned disk a leaf-disk of the same size free from poison but coated with a solution of gum arabic applied by means of a camel's hair brush.

The leaf sandwiches thus prepared were held in a paper clip, which was inserted in a split cork, and presented to the adult beetles in 6 cm. Stender dishes. On the bottom of each dish was a moistened blotting paper to prevent the leaf sandwiches from shrinking through the evaporation of

water from the leaf tissue. The beetles were given the sandwiches in the late afternoon, preferably about sundown, since feeding would start rather shortly after this time. Contrary to the usual procedure, the beetles were allowed to eat as much of the sandwich as they wanted. This made it necessary to apply each poison at various rates to different groups of leaves so that lethal, intermediate, and sublethal doses could be obtained. The following morning the partly-eaten sandwiches were superimposed upon an 18 mm. diameter circle drawn on square millimeter cross-section paper. The exact area eaten by the individual beetles was determined by counting the squares of this circle exposed by the portion of the sandwich eaten away. Each beetle was weighed on a chainomatic balance and placed in a 20 x 50 mm. vial. Moist soil and a small piece of elm leaf were added and the end of each vial was stoppered with a cork. The vials were then placed in the screened insectary, and at intervals of 24, 48, 72 and 96 hours the number of dead and living beetles was determined. A beetle that could not move its legs was considered dead.

A control group of insects was used each time a group was poisoned. These were fed sandwiches glued together with gum arabic. In a total of 467 controls a mortality of 8.6 per cent was found.

DISCUSSION

The results of the experiments with acid lead arsenate, cuprous cyanide, sodium fluoride, sodium fluosilicate, and paris green are summarized in table 1.

With acid lead arsenate, it was found that the M.L.D. for female June beetles was 0.14 mg./gram and half this amount, or 0.07, for males. Campbell (1930), using the fourth instar larvae of the silkworm (*Bombyx mori* L.), obtained an M.L.D. of 0.09 mg./gram.

Paris green proved to be more toxic than lead arsenate to the June beetles. For females the M.L.D. was 0.08 mg./gram and for males 0.04 mg./gram. Richardson and Haas (1932) estimated the M.L.D. of paris green to the larva of *Leptinotarsa decimlineata* (Say) as being less than 0.10 mg./gram, and twice that amount for the grasshopper, *Melanoplus femur-rubrum* (Deg.).

Cuprous cyanide showed a wide variation in its toxicological value upon males and females of the June beetle; an M.L.D. of 0.04 mg./gram was found for the former and 0.14 mg./gram for the latter. The M.L.D. of the males corresponded to that of the fourth instar silkworm (*Bombyx mori* L.), which is reported by Campbell and Filmer (1929) as being 0.04 mg./gram; whereas, the M.L.D. of females used in these experiments was rather close to that of the differential grasshopper (*Melanoplus differentialis* Thomas), an M.L.D. of 0.11 to 0.13 mg./gram as determined by Richardson and Thurber (1933).

With sodium fluoride an M.L.D. of 0.16 mg./gram was obtained with males and 0.29 mg./gram with females. These figures are both considerably higher than the M.L.D. of 0.11 mg./gram of this compound for the differential grasshopper as reported by Richardson and Thurber (1933).

Sodium fluosilicate was approximately twice as toxic to the male June beetle as to the females. The M.L.D. in the case of the former was found to be 0.21 mg./gram and with the latter 0.44 mg./gram. Previous to this Richardson and Haas (1932) obtained a value of 0.16 mg./gram with

TABLE 1. Toxicity of various stomach poisons to *Phyllophaga implecta* (Horn.) showing dosages in mg./gram body weight

| Compound | Paris green | | Lead arsenate | | Cuprous cyanide | | Sodium fluosilicate | | Sodium fluoride | |
|--------------------------------|-------------|-----------|---------------|-----------|-----------------|-----------|---------------------|-----------|-----------------|-----------|
| | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ |
| Lethal zone dosage range | 0.39-0.29 | 0.31-0.07 | 1.00-0.25 | 0.41-0.12 | 0.66-0.21 | 0.21-0.08 | 1.17-0.82 | 1.02-0.54 | 1.63-0.59 | 1.22-0.26 |
| Number insects | 13 | 12 | 10 | 15 | 19 | 11 | 17 | 23 | 21 | 14 |
| Intermediate zone dosage range | 0.19-0.02 | 0.06-0.01 | 0.24-0.03 | 0.11-0.02 | 0.20-0.03 | 0.77-0.01 | 0.81-0.10 | 0.48-0.09 | 0.57-0.06 | 0.25-0.04 |
| Dead | 45 | 17 | 18 | 18 | 33 | 17 | 18 | 30 | 25 | 18 |
| Recovered | 41 | 25 | 23 | 13 | 22 | 25 | 16 | 24 | 21 | 23 |
| M.L.D. | 0.08 | 0.04 | 0.14 | 0.07 | 0.14 | 0.04 | 0.44 | 0.21 | 0.29 | 0.16 |
| Median dose where killed | 0.08 | 0.04 | 0.16 | 0.07 | 0.16 | 0.05 | 0.44 | 0.22 | 0.30 | 0.17 |
| Median recovery dose | 0.08 | 0.04 | 0.12 | 0.07 | 0.13 | 0.04 | 0.44 | 0.20 | 0.27 | 0.16 |
| Sublethal zone dosage range | 0.02-0.01 | 0.01-0.00 | 0.03-0.01 | 0.02-0.01 | 0.03-0.01 | 0.01-0.00 | 0.09-0.02 | 0.08-0.01 | 0.04-0.01 | 0.03-0.00 |
| Number insects | 10 | 17 | 16 | 9 | 11 | 15 | 14 | 26 | 18 | 16 |

this poison when fed to the red-legged grasshopper, *Melanoplus femur-rubrum* (Deg.).

With the three other compounds, namely calcium arsenate, commercial manganese arsenate, and rotenone, an M.L.D. was not obtained. It was found that enough manganese arsenate could not be dusted on a leaf to produce a lethal dose under the conditions of this experiment. A 5 per cent mixture of rotenone in starch did not prove to be a lethal dose, even when the maximum quantity that would stay on a leaf was applied, so for this reason experiments were discontinued as being economically impractical. Calcium arsenate in doses ranging from 0.0012 mg./gram to 4.99 mg./gram produced only a 24 per cent mortality in 177 beetles. Manganese arsenate in doses from 0.0173 to 2.36 mg./gram produced a 7 per cent kill in 169 individuals. Rotenone doses ranging from 0.0053 to 0.386 mg./gram resulted in a 4 per cent mortality out of 83 beetles.

A study of table 1 reveals that in each instance there is a distinct difference in the M.L.D. value with each poison between male and female June beetles. The widest variation between the two sexes occurs in the case of cuprous cyanide, which was found to be three times as toxic to males as to females. With the other poisons the value of the M.L.D. in the case of females is roughly twice that for the males.

Table 2 shows a comparison of the five poisons as to their relative toxicity and as to the relative amounts of poisoned leaf eaten by the beetles. Lead arsenate was considered as equal to 1.00 in both cases and the comparisons were made on that basis. It will be observed that sodium fluosilicate was eaten by the beetles most readily. However, it should be noted that sodium fluosilicate was the least toxic and paris green the most toxic of the insecticides (table 2). The figures obtained for the different compounds compared on this basis are of value in showing the repellent action of the individual poisons.

The average weight of 138 females was 0.36 grams, with a range from 0.16 to 0.64 grams. A group of 143 males weighed from 0.19 to 0.47 grams with a mean of 0.27 grams.

TABLE 2. Showing the relative toxicity of the compounds tested and the relative amounts eaten by the beetles, assuming lead arsenate to females as equal to 1.00

| Compound | Paris green | | Lead arsenate | | Cuprous cyanide | | Sodium fluosilicate | | Sodium fluoride | |
|--|-------------|------|---------------|------|-----------------|------|---------------------|------|-----------------|------|
| | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ |
| Sex | | | | | | | | | | |
| Relative toxicity $\text{PbHAsO}_4 = 1$ (to ♀'s) | 1.75 | 3.50 | 1.00 | 2.00 | 1.00 | 3.50 | 0.32 | 0.66 | 0.48 | 0.85 |
| Relative amount of poisoned leaf eaten $\text{PbHAsO}_4 = 1$ (to ♀'s) | 0.12 | 0.10 | 1.00 | 0.94 | 0.41 | 0.36 | 1.62 | 1.57 | 1.35 | 1.21 |

SUMMARY

Adults of the June beetle, *Phyllophaga implicita* (Horn) were used as experimental insects in an effort to determine the M.L.D. values of eight stomach poisons. Values were successfully found for five of the compounds tried, namely: paris green, acid lead arsenate, cuprous cyanide, sodium fluosilicate, and sodium fluoride.

A wide variation was noted between the amounts of poison required to kill males and females of this beetle. It was found necessary, therefore, to determine an M.L.D. value of each poison for each sex. Males in every instance were killed more readily than females. Paris green proved to be the most toxic to females in mg./gram of body weight whereas paris green and cuprous cyanide were equally toxic to males. On the other hand, sodium fluosilicate proved to be least toxic to both females and males as determined on the basis of mg./gram of body weight. Females ate the poisons a little more readily than the males in every instance. The five insecticides can be arranged in the following order from the standpoint of getting the beetles to eat them most readily—sodium fluosilicate, sodium fluoride, acid lead arsenate, cuprous cyanide, and paris green (table 2).

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THE PENETRATION OF GASEOUS PYRIDINE, PIPERIDINE AND NICOTINE INTO THE BODY OF THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA* L.¹

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The cuticula of insects offers a barrier to most injurious substances which these animals encounter in their environments. Possessed of a structure and composition which afford great resistance to many chemical agents, it, nevertheless, is permeable to certain substances, including carbon dioxide and a number of inorganic and organic poisons.

This investigation attempts to establish the penetration of pyridine, piperidine and nicotine into the body of an insect from saturated atmospheres of their vapors in air. Information is also presented on the speed of entrance and the quantity of the compounds which enters the body within a given time; and an attempt is made to correlate speed of entrance with the vapor concentration and certain other known physical characteristics of the compounds. Finally, some facts are presented concerning the distribution of the compounds in the tissues.

A preliminary account of this investigation has already appeared (22). The following more detailed report presents a part of the results which have been obtained to date.

HISTORICAL

Concerning the physiological action of pyridine upon invertebrates, comparatively little is known except that it is mildly toxic to certain arthropods (20). Recently it has been found that if the oriental cockroach (*Blatta orientalis*) is exposed to pyridine gas, it yields little blood, though the cell content and process of blood coagulation are apparently normal (26). But the unpublished work of Robert A. Fisher (1935) indicates a possibly significant increase in the blood cell count of this insect following death from pyridine vapor.

Upon vertebrates, M'Kendrick and Dewar (18) observed that pyridine was the least active of a series of pyridine derivatives, including piperidine and nicotine. Brunton and Tunnicliffe (3) found that the action of pyridine on the frog and the guinea pig was confined to the sensory part of the nervous system; although in small doses it exerted a stimulating, and, in large doses, a direct paralyzing action upon cardiac muscle. deCaro (7) injected pyridine and some related compounds into the lymph sac of the frog. Acting predominantly on muscle and feebly on nervous tissue, it was the least effective of the group. From kymograph tracings obtained with sartorius muscle of the frog, Velej and Waller (31) calculated that pyridine had about one-tenth the toxic power of nicotine and

¹ A contribution to the action of compounds containing the pyridine nucleus upon insects.

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one-fifth that of piperidine. Shaw (25) found that pyridine was readily absorbed by the erythrocytes of the domestic fowl.

Although piperidine has been recognized as more toxic than pyridine to certain arthropods (20, 28), its physiological action on invertebrates has received little attention. It was established by M'Kendrick and Dewar (18) that piperidine exerted a more intense physiological action than pyridine upon a number of vertebrates. Heinz (13) found piperidine similar to pyridine in its action on the frog, but more intense. The free base of piperidine was more energetic in its action on the cornea and conjunctiva of the rabbit and the nerve endings in frog skin than was piperidine hydrochloride. The results of Veley and Waller (31) show that piperidine is about half as active as nicotine on frog muscle and that it has five times the toxic power of pyridine. According to deCaro (7) piperidine acts more energetically than pyridine upon nerve and muscle.

In an early account of the action of nicotine on invertebrates, Greenwood (10) concluded that the toxic effect on an organism was determined by the degree of development of its nervous system, unicellular organisms therefore being least affected. On the ciliate, *Chilodon megalotrocha*, Kriz (15) has shown, however, that nicotine exerts a selective action, depressing especially the posterior cilia and those of the left side.

Nicotine paralyzes the central nervous system and visceral ganglia of Mollusca and marine arthropods but does not paralyze the motor nerve endings of skeletal or visceral muscle (4). Boyer and Cardot (2) found that the isolated heart of the snail, *Helix pomatia*, was extremely sensitive to nicotine. Fries (8) failed to obtain reversal of galvanotropic responses of the isopod, *Asellus communis*, by the action of nicotine. He makes the following statement of interest here: "Although impermeability of the exoskeleton may be a factor in the failure of isopods to show any reaction to atropine and caffeine, clearly, nicotine at least can penetrate." The author does not show how penetration was demonstrated.

The literature of economic entomology is replete with references to the toxicity of nicotine to insects. This alkaloid has become almost a specific for the control of aphids and certain other small insects. For many large insects and some small species, however, its toxicity is much lower. On the other hand, the physiological action of nicotine upon insects has received much less attention.

McIndoo (17) made a study of the action of nicotine upon various species of insects and reviewed the more pertinent literature. He described the responses of insects in contact with the vapors and solutions of nicotine, and sought to establish the presence of the alkaloid in certain tissues of the insects. Of greatest interest for the present investigation are his experiments in which attempts were made to precipitate the alkaloid by means of phosphomolybdic acid in the tissues of the insects immediately after death from nicotine poisoning. The nicotinized insects were sectioned and certain tissues were examined for the presence of nicotine precipitates. Precipitates were reported in the tracheae and in the ventral ganglia which he believed were reaction products of nicotine and phosphomolybdic acid. McIndoo concluded that nicotine in solution does not pass into the integument or the tracheae of insects, whereas, nicotine vapor does pass into the tracheae and is widely distributed in the tissues. The toxic action results, he thought, from paralysis which travels along the ventral cord from the abdomen to the brain.

Schellhase (24) produced convulsive responses, quickly followed by death, with weak solutions of nicotine applied to the neck stump of decapitated hippoboscids. Becker (1) made a similar study of alkaloids upon grasshoppers, injecting solutions of the compounds into the pericardial cavity, into the head ampullae or into the intestine of decapitated insects. Nicotine brought about a tonic cramp in the jumping leg.

Richardson and Shepard (21) observed that the speed of toxic action of aqueous nicotine solutions for mosquito larvae, *Culex pipiens*, was directly related to the concentration of the undissociated nicotine molecules. Under the experimental conditions, penetration of the nicotine probably took place largely through the mouth, into the digestive tract and thence into the tissues, although penetration through the integument was also probable. Macht and Craig (16) also observed large differences in the toxicity of nicotine base and nicotine salts for a number of invertebrate and vertebrate animals.

Portier (19) placed strong nicotine solutions upon the antennae of adults of *Vanessa atalanta* and *Satyrus actea*. The alkaloid penetrated the nerves and probably the tracheae in these appendages. Hartzell and Wilcoxon (12) report experiments which tend to show that nicotine is able to penetrate the integument of the tobacco worm, *Phlegethontius quinque-maculata*.

Shull, Riley and Richardson (26) observed no visible effect of nicotine on the process of blood coagulation in the oriental cockroach, *Blatta orientalis*, or upon the blood cells.

The principal features of the action of nicotine on vertebrates as understood at present, are well described by Cushny (6). Death of the animal is said to result from paralysis of the respiratory center. Thomas and Franke (29), however, contend that failure of the respiratory mechanism results primarily from the curare-like paralysis of the respiratory muscles.

MATERIALS

The insects used in this investigation were largely adults of the American cockroach, *Periplaneta americana*, the average weight being 0.948 gram; the few younger individuals employed were in the late nymphal instars.

The chemicals employed in the tests were high-grade products. The pyridine was redistilled from a reagent grade and collected between 112° and 113° C. (740 mm. pressure). The piperidine was of reagent grade, and was kept in an anhydrous condition by contact with solid potassium hydroxide. The nicotine was a redistilled product which tested 99.32 per cent nicotine.

The reagents by means of which the compounds were detected and quantitatively estimated were three in number: Wagner's reagent, a solution containing iodine and potassium iodide, proved most useful to confirm detection of pyridine; phosphomolybdic acid reagent, a 10 per cent solution of phosphomolybdic acid, which contains 1 per cent by volume of concentrated nitric acid, was used to estimate the three compounds, whereas gold bromide reagent (a solution containing equal quantities of $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ and NaBr in water) was used only to confirm the presence of piperidine and nicotine. The formulas for the preparation of these reagents are given by Fulton (9).

METHODS

The insects were exposed to the vapors at 30° C. in an ordinary glass bottle of 250 cc. capacity, the small wire cage which held the insects being suspended from a hook inserted into the shellacked cork stopper. An almost saturated atmosphere of the compound in the bottle was assured by an excess of the compound on the bottom.

Since these bases are volatile in steam, the following procedure was used for their determination in whole insects. Three insects were weighed and exposed to a gas for a definite time; they were then removed, washed in a stream of distilled water, placed in a flask with 20 cc. of absolute alcohol acidified with tartaric acid, cut into fragments with scissors, transferred to a 200 cc. Kjeldahl flask and refluxed for about 10 minutes. The condenser was a test tube which fitted snugly into the neck of the flask. After the removal of the alcohol, the sirupy residue was dissolved in 20 cc. of distilled water. Filtration was unnecessary here, for blank determinations of untreated tissue showed that no material was present which would pass into the distillates to interfere with the reagents used in detecting the toxic compounds. This acidified mixture of extract and extracted tissue was made alkaline with dilute potassium hydroxide solution; then steam was passed through it until 100 cc. of the distillate were collected in a weak tartaric acid solution. Previous tests showed that the compounds were completely recovered in this volume of distillate. The distillates were concentrated to the desired volume on a water bath aided by a current of filtered air. Usually at least three independent determinations were made.

When the compounds were to be determined in the various tissues of the insects, the procedure was varied somewhat. The tissues desired were dissected into tared weighing bottles. Muscular tissues were obtained from the leg and wing muscles, the average moist weight of muscular tissue from a single roach being 0.085 gram; the digestive tract (average weight 0.126 gram) included the whole alimentary canal; the nerve tissue (average weight 0.007 gram) included the entire ventral nerve cord but not the brain. The fat body tissue (average weight 0.069 gram) was removed directly from the body cavity; the cuticula³ (average weight 0.423 gram) was the entire exoskeleton with most of the abdominal muscles and fatty tissue removed. Usually a smaller total weight of material had to be used for the determination of the compounds in the tissues than for the determinations in the whole insects.

Whatever the toxic compound used, the concentrated distillates were first tested with the phosphomolybdic acid reagent. With nicotine, this reagent gives an evident turbid amorphous precipitate in a solution containing 0.002 gram per 100 cc. of solution. Gold bromide was used to confirm the presence of nicotine. Piperidine gives with phosphomolybdic acid reagent a crystalline precipitate of characteristic, small, irregular grains easily discernible in solutions containing 0.05 gram of piperidine per 100 cc. of solution. Gold bromide precipitates large, dark-brown, branched crystals at concentrations of piperidine considerably below the

³ The term cuticula is used here in a somewhat loose sense, to include the various layers of the true cuticula, the underlying epidermis and the basement membrane. (Cf. Snodgrass, 27.)

limits detected by the phosphomolybdic acid reagent. With concentrations of 0.006 gram per 100 cc., pyridine yields lens-shaped crystals with the phosphomolybdic acid reagent. Wagner's reagent, somewhat more sensitive (0.004 gram of pyridine in 100 cc. or less) was used to confirm the presence of this compound. The crystals, brown, flat and characteristically notched, are very diagnostic.

After a concentrated distillate was tested to determine the presence of the toxic compound, a 1 cc.-aliquot of it was diluted with an equal volume of distilled water; this solution was then diluted to form a series of solutions each containing one-half the concentration of the solution above it. To a drop from each solution on a glass slide was added a drop of the phosphomolybdic acid reagent. The solution, which gave a faint but easily discernible precipitate (with nicotine, equivalent to that of a 0.002 per cent nicotine solution), was taken as the limiting dilution of the compound. With a knowledge of the weight of the original insect tissue extracted, the volume of the original distillate, degree of dilution of the distillate and concentration of the toxic compound at the limiting dilution, it was possible to express the approximate quantity of toxic compound absorbed in milligrams per gram of insect tissue. The presence of the compound in the diluted distillate was further confirmed by means of another reagent as indicated above.

Not all of the reagents were employed at the exact limits of detection of the particular toxic compound. The "end-point" selected was one at which detection was certain and comparisons could be made without great difficulty. This required some experience and repeated verification with solutions of known concentration of the toxic compounds. The quantitative values, of greater worth as comparative measures than as absolute measures of the compounds present in the insect, are, however, considered adequate for the present study.

RESULTS

The average concentration of pyridine in the bodies of cockroaches (fig. 1) after being exposed to the vapors for 5 minutes was 0.4 mg./g. body weight; in 20 minutes 2.0 mg./g., in 40 minutes 2.9 mg./g., and in 60 minutes 2.9 mg./g. In 40 minutes, about 22 per cent of the insects were dead, and in 60 minutes 73 per cent. Thus 50 per cent of the roaches died in about 51 minutes when the body contained pyridine at a concentration of 2.9 mg./g.

In the experiments in which body parts and tissues were tested for the toxic compounds, pyridine did not seem to exhibit great selectivity. However, even in the first 5 minutes, relatively large quantities of the base were found in the ventral nerve cord, and the concentration increased up to 20 minutes (fig. 6). The cuticula (fig. 2) received only a small quantity of pyridine in 5 minutes, though the amount was greatly increased at 60 minutes. The fat body (fig. 5) showed only a small quantity in 5 minutes, but it was increased in 60 minutes to a value comparable with that in the digestive tract (fig. 4). Even after 60 minutes, the muscle (fig. 3) had taken up only a small quantity of pyridine.

The average concentration of piperidine in whole cockroaches exposed to the vapors for 5 minutes was 0.6 mg. per gram of body weight; for 10 minutes, 1.0 mg./g.; for 16 minutes, 1.1 mg./g., and for 20 minutes

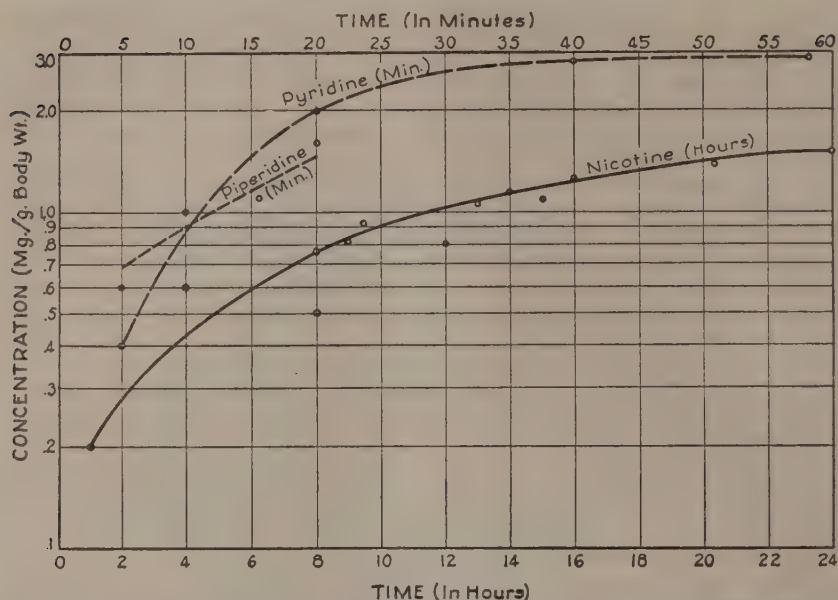


Fig. 1. Relative time course of entrance of pyridine, piperidine and nicotine into the entire American cockroach, *Periplaneta americana*, from saturated atmospheres of their vapors in air. For pyridine and piperidine the abscissas indicate minutes, for nicotine hours.

1.7 mg./g. (fig. 1). Thirty-six per cent of the cockroaches were dead in 10 minutes, 70 per cent in 16 minutes and 82 per cent in 20 minutes. About 50 per cent of the roaches had died in 12.5 minutes when the body contained a piperidine concentration of about 1 mg./g.

During the 20 minute exposure, there was a very great accumulation of piperidine in the muscle (fig. 3). The cuticula and the ventral nerve cord received a similar amount. At all time levels, the fat body showed the lowest concentration of piperidine (fig. 5).

The average concentration of nicotine found in entire cockroaches after 60 minutes exposure was 0.2 mg. per gram of body weight; after 8 hours about 0.77 mg./g.; after 13 hours about 1.05 mg./g.; after 16 hours about 1.3 mg./g., and after 20.3 hours about 1.5 mg./g. (fig. 1). About 50 per cent of the insects were dead in 14.3 hours when the body contained about 1.2 mg./g. of nicotine.

In no tissues examined was there such a high concentration of nicotine as in the cuticula. The muscle, fat body, digestive tract and nerve cord in 900 minutes showed about equal quantities of this compound.

DISCUSSION OF RESULTS

Pyridine, piperidine and nicotine are representatives, respectively, of three series of homologous compounds. In structure and in most physical characteristics, pyridine and piperidine are more alike than either is to nicotine and they resemble each other more in their physiological action and toxicity.

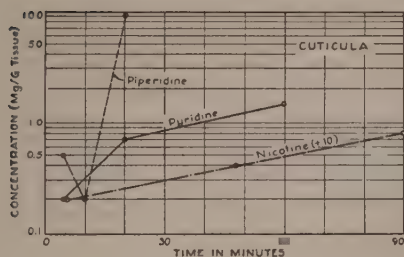


Fig. 2. Rate of entrance of the three bases into the cuticula of the cockroach. For nicotine the time is divided by 10.

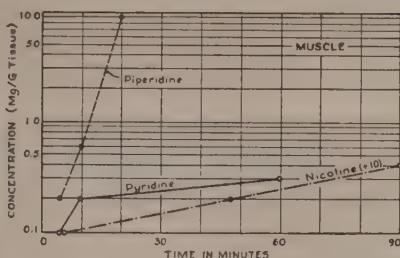
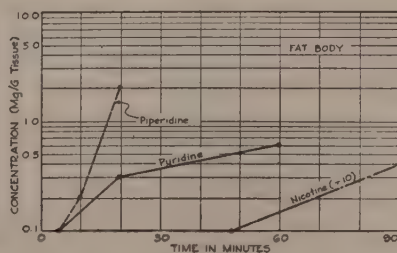
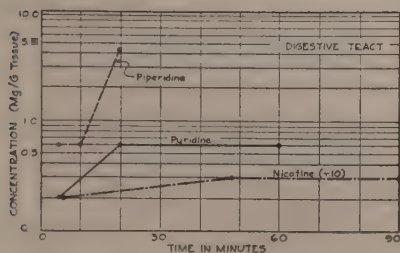


Fig. 3. Rate of entrance of the three bases into the muscles of the wings and legs. For nicotine the time is divided by 10.

The following table furnishes a comparison of these compounds with respect to vapor concentration in air and toxicity to the cockroach.

| | Nicotine (11) | Pyridine (32) | Piperidine (23) |
|---|------------------|------------------|--------------------|
| Vapor concentration in air at 30° C., mg./liter | 0.27 | 111 | 173 |
| Concentration in body when about 50 per cent of the insects were dead, mg./gram | 1.2 | 2.9 | 1.0 |
| Approximate time in minutes to kill about 50 per cent of the insects | 860 | 51 | 12.5 |

The ratio of vapor concentration calculated from the above data (nicotine:pyridine:piperidine) is 1:411:641. On the basis of the concentration present in the bodies of the cockroaches when approximately 50 per cent of the insects were dead the ratio for the compounds taken in the same order is 1:2.4:0.9. The numerical order of piperidine and pyridine is here reversed and nicotine and piperidine are about equal, that is the roaches have absorbed less piperidine and nicotine than pyridine, when 50 per cent are dead. However, in 12.5 minutes piperidine has killed 50 per cent of the insects and nicotine and pyridine have killed none. In the



Figs. 4 and 5. Rate of entrance of the three bases into the digestive tract tissues and the fat body. For nicotine the time is divided by 10.

case of the insects exposed to nicotine the outside concentration is not large, so accumulation in the body of the amount necessary to kill 50 per cent of the insects (that is, 1.2 mg./gm.) proceeds slowly; on the other

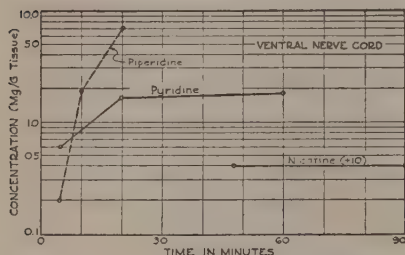


Fig. 6. Rate of entrance of the three bases into the ventral nerve cord. For nicotine time is divided by 10.

hand, pyridine and piperidine with their much greater vapor concentrations offer an almost infinitely large quantity of toxic gas from which the insects accumulate in a much shorter time the requisite amounts for death. From the above data the relative toxicity is probably best expressed by the ratio of the product, vapor concentration \times concentration in the body to kill 50 per cent \times the time necessary to kill 50 per cent; for nicotine, pyridine and piperidine, this ratio is 1:59:8. The sequence of the three compounds arranged in

decreasing order on the basis of this toxicity ratio is: nicotine $>$ piperidine $>$ pyridine.

In water, these compounds are all highly soluble. Their toxicities do not follow their solubilities in this medium.

The molecular weights of the three compounds are: nicotine 162.12, pyridine 79.05, piperidine 85.09; when taken in this order the molecular weights arrange themselves in the ratio 1:0.488:0.532. There is a certain relation between the order of the molecular weights and the toxicity, but the difference in the toxicity between pyridine and piperidine is much greater than the difference between their molecular weights.

The dissociation constants of these compounds in water are: piperidine 1.10×10^{-3} (30); nicotine 9×10^{-7} (5); pyridine 1.4×10^{-9} (14). Piperidine is relatively a strong base, being stronger than ammonium hydroxide ($K = 1.8 \times 10^{-5}$). Pyridine is a very weak base, whereas nicotine occupies an intermediate position. The order of toxicity of these bases is not the order of their dissociation constants. Neither does the speed of entrance follow this order, for in the first 20 minutes of exposure, piperidine and pyridine enter the body at nearly the same rate. Apparently the more dissociable (ionic) piperidine is more toxic and as permeable to this insect as the relatively undissociable pyridine.

A study of the distribution of the compounds in the various body parts and tissues shows a number of differences. Nicotine, for example, steadily accumulates in the cuticula even after its concentration in the digestive tract tissues and the ventral nerve cord have reached apparent equilibrium. The quantities of nicotine in the muscle and fat body also increase, but the final concentrations reached are not so large.

McIndoo (17) has contended that nicotine enters the insect through the tracheae and thence passes into the tissues. The present data do not support this view. Furthermore, it has been shown (22) that nicotine, piperidine and pyridine vapors are able to penetrate directly into the cuticula of several species of insects. Cockroaches were exposed to the vapors by being fastened to the cork stoppers of gas bottles with only a part of one anterior wing sealed off in contact with the gas. Sufficient quantities of these compounds to give good quantitative values were

found in the tissues of the exposed insects. Corn ear worm larvae and grasshoppers, similarly sealed so that only a portion of the cuticula was in contact with the toxic vapors, also demonstrated the penetration of these compounds directly through the cuticula. Details of these experiments will soon be published.

Piperidine accumulated chiefly in the cuticula, muscular tissue and ventral nerve cord. In 20 minutes, the concentrations in these three tissues were about equal. The digestive tract and fat body received smaller amounts of piperidine. Apparently the accumulation of piperidine in the ventral nerve cord is more rapid than in any of the other tissues. The evidence here likewise points to the importance of penetration through the cuticula. The tissues nearest the cuticula, namely the ventral nerve cord and the leg and wing muscles, contain the greatest quantity of piperidine; those tissues farthest removed from the cuticula (fat body, digestive tract) the least. A gradient is therefore established from the cuticula to the innermost tissues. Such a gradient would probably not be present if these tissues were absorbing piperidine only from the blood, which, in turn, obtained it from the tracheae. This is shown in a less striking degree by pyridine.

After 60 minutes, pyridine is found in approximately the same concentration in both cuticula and ventral nerve cord, though in the latter tissues the rate of accumulation is higher. The accumulation in the muscle, digestive tract and fat body is similar, with least appearing in the muscle. This is interesting in view of deCaro's conclusion (7) that in vertebrates pyridine is a muscle poison. However, the concentration of a compound in a tissue may not be closely correlated with the physiological action which it produces.

The authors wish to acknowledge the support given by a grant from the Rockefeller Fluid Research Fund, which made this project possible; and the cooperation of Mr. C. C. Fulton, who kindly furnished certain information on the sensitivities of precipitating reagents for the toxic compounds. They are also indebted to Dr. L. O. Ellisor for assistance with certain experiments.

SUMMARY AND CONCLUSIONS

The American cockroach, *Periplaneta americana* (L.) was treated with pyridine, piperidine and nicotine in the gaseous state. Entire insects, parts and tissues were extracted and the compounds were detected and their concentrations were determined in the extracts.

On the basis of the products, outside concentration \times concentration in the body for 50 per cent mortality \times time for 50 per cent of mortality, the compounds show the following order of toxicity: nicotine $>$ piperidine $>$ pyridine. This is the order of their toxicity and of their known physiological action upon vertebrate animals.

The approximate concentrations in the bodies of the cockroaches when 50 per cent were dead and the times for 50 per cent mortality are as follows: pyridine 2.9 mg./g. in 51 minutes; piperidine 1.0 mg./g. in 12.5 minutes, and nicotine 1.2 mg./g. in 860 minutes.

These compounds appear to enter the body largely through the cuticula.

The ventral nerve cord does not take up as much nicotine as the cuticula and no more than the large muscles, fat body and the tissues of the digestive tract.

The ventral nerve cord accumulates as much pyridine as the cuticula; the digestive tract tissues and the fat body less. The muscle showed only a small amount of pyridine.

Likewise, the ventral nerve cord and the cuticula showed about equal quantities of piperidine. There was a markedly large amount of piperidine in the muscles, whereas a relatively smaller amount was found in the fat body.

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STUDIES ON *ALCALIGENES VISCOSUS*^{1, 2}

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Alcaligenes viscosus is one of the well-known organisms found in dairy products. It was apparently first isolated by Adametz (1), who obtained it from water and named it *Bacillus lactis viscosus*; because of its action on milk this investigator believed the organism responsible for ropiness in dairy products. Since the studies of Adametz, various other investigators have obtained *A. viscosus* from many outbreaks of ropiness in milk, cream and other dairy products.

It appears that most of the cultures of *A. viscosus* which have been identified have come from outbreaks of ropiness in dairy products. This defect suggests one of a relatively few species of organisms, just as do a number of the other defects of milk and its derivatives, and thus the identification of the causative organism is comparatively easy. It would be expected, however, that if *A. viscosus* is rather frequently found in milk and cream in numbers sufficient to cause ropiness, it often would be present in smaller numbers and so should be encountered in normal milk and cream.

One of the little known characters of *A. viscosus* is its ability to hydrolyze fat which is readily evident with the Nile-blue sulfate technique (4). This character suggests the species may be important from the standpoint of the lipolytic organisms in dairy products.

In a study of the fat-splitting bacteria in normal and abnormal (but not ropy) milk, cream and similar products, a large number of organisms were isolated. Thirty-six of the cultures obtained showed the characters of *A. viscosus*, including the production of ropiness in milk. These cultures were studied in considerable detail.

SPECIAL CHARACTERS OF *A. VISCOSUS*

Action on carbohydrates, etc. In general, carbohydrates, etc., in bouillon were attacked only slightly if at all by the cultures of *A. viscosus* isolated. A few of the cultures produced acid, but no gas, from certain of the sugars and later reversed the reaction. Ayres, Rupp, and Johnson (2) studied various alkali-forming bacteria from milk and suggested that the alkaline reaction is due to the oxidation of the salts of organic acids present in milk or beef-extract broth to alkaline carbonates. In order to prevent an alkaline fermentation they suggested three synthetic media containing dextrose, sodium ammonium phosphate, and potassium chloride in various concentrations. The 36 cultures of *A. viscosus* were inoculated into two of the media of Ayres, Rupp, and Johnson. In one (medium A), none

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of the cultures brought about an acid reaction, while in the other (medium C), which contained half the amount of sodium ammonium phosphate and double the amount of potassium chloride, 12 of the 36 cultures produced an acid reaction. With the low fermenting powers of *A. viscosus*, it appears that the action on sugars and comparable materials is of little value in identifying this species.

Hydrolysis of fat. All 36 of the cultures hydrolyzed fat when tested with the Nile-blue sulfate technique (4), using cottonseed oil and beef infusion agar; the cultures were spotted on the agar, and fat hydrolysis was quickly evident. There was considerable variation in the types of lipolysis (3) produced by the various cultures. Thirty-two of the cultures hydrolyzed all of the globules beneath the growth, while four hydrolyzed only a part of them. Twenty-four of the cultures hydrolyzed the fat for a considerable distance beyond the edge of the colony, while twelve hydrolyzed only beneath the colony.

Hydrolysis of simple tri-glycerides. The 36 cultures differed somewhat in their action on various simple tri-glycerides dispersed in beef infusion agar containing Nile-blue sulfate. All of them hydrolyzed tri-butyrin and tri-olein, while none of them hydrolyzed tri-myristin, tri-palmitin, and tri-stearin. Action was variable on tri-propionin, tri-n-valerin, tri-isovalerin, tri-caproin, tri-heptylin, tri-caprylin, tri-caprin, and tri-laurin.

Action in cream. Each of nine cultures of *A. viscosus* was inoculated into a flask of sterile cream and the cream incubated at 21° C. After seven days all of the cultures had developed rancidity; there was considerable variation in the extent of the defect.

Action in butter. Each of seven cultures of *A. viscosus* was inoculated into a pint of cream that had been pasteurized at 82° C. for 15 minutes and the cream churned. The unsalted butter was held at 21° C. The numbers of organisms in the butter after six days were rather high, varying from 19,200,000 to 254,000,000 per ml. Three of the cultures produced a definite rancidity in the butter, while two brought about a ropiness in the body with no flavor defect. The remaining two cultures did not appear to affect the butter in any manner.

Production of acetylmethylcarbinol plus diacetyl. Four of the cultures were studied for their ability to produce acetylmethylcarbinol plus diacetyl in skim milk. When 200 gm. portions of the cultures that had been incubated five days at 21° C. were steam distilled with ferric chloride and the distillates collected in a solution of hydroxylamine hydrochloride and sodium acetate, no nickel dimethylglyoximate was obtained when nickel chloride was added.

DESCRIPTION OF *A. VISCOSUS*

MORPHOLOGY

(Cultures grown at 21° C.)

Form and size. Rods; 0.6 to 1.0 by 0.8 to 2.6 μ when grown one day on beef infusion agar. Cells that were almost spherical were frequently found in young bouillon or litmus milk cultures.

Arrangement. Singly, in pairs or short chains when grown in litmus milk.

Motility. Non-motile.

Staining reaction. Gram negative; in certain cultures there was a tendency for a few cells to retain the gram stain.

Spores. None formed.

Capsules. Produced in milk cultures.

CULTURAL CHARACTERS

(Cultures grown at 21° C.)

Agar slope. Beef infusion agar showed an abundant, white, spreading, viscid, shiny growth after one to two days.

Agar stab. In beef infusion agar there was a moderately heavy, viscid surface growth with some development along the line of inoculation.

Agar colony. On beef infusion agar, growth was evident in approximately one day, while after three to four days surface colonies were white, viscid, shiny, round with entire edge, and from 4 to 6 mm. in diameter; subsurface colonies were white, viscid, oval, and much smaller than surface colonies.

Gelatin stab. No liquefaction. There was a moderately heavy, viscid surface growth with some development along the line of inoculation.

Bouillon. A thin pellicle was formed, with turbidity and some sediment. Ropiness was generally produced.

Potato. A moderately heavy, dirty white, spreading, shiny growth developed.

Litmus milk. Ropiness was produced in litmus milk, but the extent and time required varied widely. Some cultures produced ropiness in much less than one day; others required several days, and there were various rates in between these extremes. As the ropiness increased, a clear, slimy layer of material often formed on the top of the milk. A pellicle developed on the milk; with cultures producing ropiness rapidly, the pellicle followed the development of ropiness, while with cultures producing ropiness slowly the pellicle preceded the development of ropiness. After some days the milk became distinctly alkaline. There was no coagulation or evident proteolysis. With certain cultures there was a slight reduction of the litmus at the bottom of the tube after an extended period. Cultures some days old commonly had a very characteristic odor which at first more nearly resembled fishiness but later suggested decomposed urine.

BIOCHEMICAL FEATURES

(Cultures grown at 21° C.)

Indol. Not produced.

Nitrates. Not reduced.

Hydrogen sulphide. Not produced.

Methyl red reaction. Negative.

Voges Proskauer reaction. Negative.

Action on carbohydrates, etc. Generally neither acid nor gas was formed in bouillon containing glycerol, arabinose, dextrose, levulose, galactose, maltose, lactose, sucrose, mannitol, salicin, raffinose, or inulin. A few of the cultures occasionally produced acid from glycerol, arabinose, levulose, galactose, maltose, lactose, and mannitol, but were not consistent in this respect; all of the culture producing acid from carbohydrates, etc., later reversed the reaction.

Hydrolysis of fat. Fat was hydrolyzed.

GROWTH CONDITIONS

Oxygen relationship. Aerobic.

Temperature relationship. Growth occurred at 10° C. and 20° C., while at 37° C. and 40° C. some cultures grew and others did not.

CULTURES LIKE *A. VISCOSUS* EXCEPT FOR THE FAILURE TO PRODUCE ROPINESS

The cultures of lipolytic organisms isolated from milk, cream, etc., included 25, which except for the lack of ropiness, produced the same general change in litmus milk as *A. viscosus*. An alkaline reaction developed slowly in milk, and commonly an inconspicuous pellicle formed. The morphology, staining reactions, and biochemical characters were the same as with *A. viscosus* and, except for the failure to produce ropiness in milk, bouillons, etc., the cultural characters were also the same. These cultures were regarded as non-ropy strains of *A. viscosus*. Five of the cultures which did not produce ropiness in milk when first isolated began to form ropiness after they had been through a number of transfers in milk. In this connection it is significant that considerable variation existed in the degree of ropiness produced in milk by the typical cultures of *A. viscosus*, and also in the time required to produce ropiness.

SPECIAL CHARACTERS OF THE NON-ROPY CULTURES

Action on carbohydrates, etc. The action of the non-ropy cultures on the various carbohydrates, etc., was similar to that of the typical *A. viscosus* cultures. In medium A of Ayres, Rupp, and Johnson (2) two of the 25 cultures produced an acid reaction, while in medium C, 12 cultures produced an acid reaction.

Hydrolysis of fat. All 25 of the cultures hydrolyzed fat when tested by the method employed with the typical *A. viscosus* cultures, and all of them completely hydrolyzed the fat beneath the growth. Fourteen of the cultures produced hydrolysis for a considerable distance beyond the edge of the colony, while 11 hydrolyzed only beneath the colony.

Hydrolysis of simple tri-glycerides. The cultures had the same general action on the simple tri-glycerides as the typical *A. viscosus* cultures.

Action in cream. Each of the nine cultures inoculated into sterile cream produced rancidity after incubating 7 days at 21° C.

Action in butter. Three of the cultures were examined for their action on butter, using the procedure employed with the typical *A. viscosus* cultures. One of the cultures produced a definite rancidity, while the other two had no effect on the butter.

Production of acetylmethylcarbinol plus diacetyl. The four cultures studied failed to produce acetylmethylcarbinol plus diacetyl when tested by the method used with the typical *A. viscosus* cultures.

INTERPRETATION OF THE NON-ROPY CULTURES

The cultures which are like *A. viscosus*, except for the failure to produce ropiness, are considered to be non-ropy strains of *A. viscosus*, and the designation *Alcaligenes viscosus* var. *dissimilis* is suggested for them. Both ropy and non-ropy strains are found with various bacterial species involved in the production of ropiness in milk, for example, *Streptococcus lactis*, *Streptococcus citrovorus*, species of the *Escherichia-Aerobacter* group, and *Lactobacillus bulgaricus*, so that the situation noted with

A. viscosus is in agreement with that existing with various species of bacteria.

SUMMARY

The results reported indicate that organisms which are like *A. viscosus*, except for the failure to produce ropiness in milk and other liquid media, are commonly encountered among the organisms isolated from dairy products. The designation *Alcaligenes viscosus* var. *dissimilis* is suggested for them. Some of the cultures which produced no ropiness in milk when first isolated began to form ropiness after they had been through a number of transfers in milk. The typical *A. viscosus* cultures that were studied varied widely in the extent of the ropiness developed in milk and in the time required to produce ropiness.

A description of *A. viscosus* is presented. One of the little known characters of this organism is its ability to hydrolyze fat.

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EFFECT OF TREATMENT OF OATS ON THE DEVELOPMENT OF SACCHAROGENIC AND DEXTRINOGENIC AMYLASES DURING GERMINATION

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Studies on the preparation and properties of purified enzyme from germinated wheat (7), rye (17), and corn (4) have been made. It has seemed difficult to study the enzymes of germinated oats, because the varieties of oats which could be obtained were susceptible to mold growth during germination. Since an appreciable amount of dextrinogenic enzyme accompanies mold growth, it is evident that enzyme determinations made on such grain are in error.

There are many methods for the treatment of oats which are intended to kill larvae and insects with which the grain may become infested during storage, to prevent mold growth and consequent heating of grain in bins, and to control smut and other seed diseases. It was considered advisable to make a study of some of these chemicals used in the treatment of grains to determine whether any of them would prevent mold growth during germination of oats and, at the same time, would not inhibit the activity or development of enzyme.

Grain treated with inorganic compounds, such as copper carbonate (15, 18), copper oxychloride (18), copper sulphate (27), calcium cyanide (19), or alcoholic mercuric chloride (3), could not be used for enzyme studies because copper and mercury salts are known to have an inhibitive effect upon amylase activity (10, 13, 16, 26). For the same reason, it was impossible to use metallic salts of organic compounds such as mercurated tricresols (14), mercurated furfural (28), and many similar compounds which are effective germicides for treatment of grains.

The use of gaseous insecticides was considered a possible method for sterilizing the grain against mold, because the gas could attack the mold spores and then be allowed to evaporate before the grain was germinated. In this way, the insecticide would probably have a minimum effect upon the activity and development of enzyme. Back (1, 2), reports the use of carbon disulfide, ethylene dichloride-carbon tetrachloride mixtures, and ethylene oxide-carbon dioxide mixtures as successful in control of insects in farm elevators. Duval (8), and Cotton and Roark (6) agree on the efficiency of ethylene oxide as a fumigant and recommend a method of treatment of grain with an ethylene oxide-solid carbon dioxide mixture. In the experiments described in this paper, a study was made of the development of dextrinogenic and saccharogenic enzyme in oats which have been treated with carbon disulfide, with ethylene dichloride, with a mixture of carbon tetrachloride and ethylene dichloride, and with an ethylene oxide-solid carbon dioxide mixture.

An attempt was made to use for treatment of small amounts of grain, a method corresponding to that described by Back (1, 2), Duval (8), and Cotton and Roark (6) for fumigation of grain in bins. The required

amount of carbon disulfide or of ethylene dichloride was measured into a test tube which was then dropped into a large flask of grain. The flask was closed and the contents shaken at intervals to insure contact of the grain with the gaseous insecticide. Using carbon disulfide, 0.2568 g. or 0.20 cc. of it was used on 1000 g. of grain in a four-liter flask and allowed to stand four days. Using ethylene dichloride, which was about one-fifth as toxic as carbon disulfide, 1.02 cc. of the substance was used in the four-liter flask of grain.

Another sample of the grain was treated with a mixture of three parts (by volume) of ethylene dichloride and one part of carbon tetrachloride with 1000 g. of oats. The carbon tetrachloride reduces inflammability and increases the volatility. A control sample of oats was treated with carbon tetrachloride alone.

The treatment recommended by Duval (8) was accomplished by preparing a mixture of 0.094 g. or 0.10 cc. of ethylene oxide with solid carbon dioxide and dropping this into a four-liter flask with 1000 g. of oats. The oats remained in contact with this mixture for four days.

Germination of these samples of grain showed that carbon disulfide and ethylene dichloride control mold growth effectively and permit satisfactory germination of the grain. Carbon tetrachloride does not control mold growth. The grain treated with ethylene oxide germinated without mold growth, but germination was very slow.

After treatment, the grain was placed on moist blotters and germinated at 20° C. in a germinator with temperature and humidity control. The grain was washed, dried, and ground to a fine flour. A water extract was made and tested for dextrinogenic and saccharogenic activity by standard methods for enzyme determinations (7, 23, 24, 29.) The saccharogenic activity (tables 1 and 3) is measured by determination of the mg. of maltose formed in half-hour digestion of starch dispersion at 40° C. per mg. of total solid from the enzyme extract. A measured volume of the diluted extract is used in the digestion mixtures, while another measured portion of the extract is evaporated to constant weight in determination of the weight of total solid in 5 cc. of extract. In tables 2 and 4, the results are reported as dextrinogenic activity, which is the number of mg. of enzyme (total solid from enzyme extract) required, in one-half hour digestion at 40° C., to change 5 cc. of one per cent starch dispersion to products giving no blue color with iodine.

From the results indicated in table 1, it is evident that the saccharogenic enzyme of dormant grain is influenced very little by the treatments

TABLE 1. *Influence of treatment of oats upon saccharogenic amylase*

| Treatment | Saccharogenic power ungerminated oats | Saccharogenic power germinated oats |
|--|--|--|
| Carbon disulfide | 5.25 | 24.0 |
| Carbon tetrachloride | 6.26 | 25.06 |
| Ethylene dichloride and carbon tetrachloride | 5.58 | 19.12 |
| Ethylene dichloride | 5.51 | 16.81 |
| Ethylene oxide and carbon dioxide | 4.67 | 17.29 |

TABLE 2. *Influence of treatment of oats upon the dextrinogenic amylase*

| Treatment | Dextrinogenic power ungerminated oats | Dextrinogenic power germinated oats |
|--|---------------------------------------|-------------------------------------|
| Carbon disulfide | 243 | 4390 |
| Carbon tetrachloride | 256 | 2880 |
| Ethylene dichloride and carbon tetrachloride | 271 | 1435 |
| Ethylene dichloride | 254 | 6070 |
| Ethylene oxide and carbon dioxide | 243 | 1882 |

used. The saccharogenic activity is slightly higher for the germinated grain treated with carbon disulfide and with carbon tetrachloride; and compared with these, the enzyme activity is lower for those grains treated with ethylene dichloride and with ethylene oxide-carbon dioxide. These latter treatments control mold growth effectively but inhibit somewhat the production of saccharogenic amylase during germination.

The results of a study of the effect of these treatments upon the dextrinogenic enzyme of oats is given in table 2. The dextrinogenic enzyme of dormant oats is influenced very little by the treatments used. The dextrinogenic activity is higher for the germinated grains treated with ethylene dichloride and with carbon disulfide; and compared with these, the enzyme activity is lower for the grains treated with carbon tetrachloride, ethylene dichloride-carbon tetrachloride mixtures, and ethylene oxide-carbon dioxide mixture.

These results indicate that the saccharogenic and dextrinogenic enzymes are not affected in exactly the same way by the chemical treatments used. It is evident that fumigation of the grain with ethylene oxide-carbon dioxide mixture causes an apparent slowing up of the germination of the grain and a decrease in activity for both amylases. After treatment with carbon disulfide, the germinated grain contains both

TABLE 3. *Influence of treatment of oats on development of saccharogenic enzyme*

| Time of germination (days) | Treatment with carbon disulfide | | Treatment with ethylene dichloride | | Treatment with ethylene oxide | |
|----------------------------|--------------------------------------|---------------------|--------------------------------------|---------------------|--------------------------------------|---------------------|
| | Mg. Cu ₂ O per 2 cc. ext. | Saccharogenic power | Mg. Cu ₂ O per 2 cc. ext. | Saccharogenic power | Mg. Cu ₂ O per 2 cc. ext. | Saccharogenic power |
| 0 | 41 | 5.1 | 36.9 | 5.1 | 32.3 | 4.8 |
| 1 | 58.8 | 7.0 | 51.6 | 5.3 | 52.5 | 6.3 |
| 2 | 122.8 | 13.7 | 73.5 | 8.1 | 80.5 | 8.0 |
| 3 | 204.4 | 17.3 | 199.6 | 19.7 | 98.6 | 6.0 |
| 4 | 256.2 | 17.7 | 191.2 | 15.2 | 67.4 | 6.9 |
| 5 | 288.0 | 19.1 | 176.3 | 16.8 | 216.6 | 15.4 |
| 6 | 300.8 | 23.2 | 323.2 | 25.8 | 266.4 | 22.0 |
| 7 | 421.2 | 23.9 | 415.0 | 20.5 | 309.2 | 17.3 |
| 8 | 562.0 | 17.0 | 492.4 | 16.8 | 275.2 | 24.4 |
| 9 | 466.8 | 13.5 | 454.2 | 15.4 | 520.6 | 16.3 |
| 10 | 572.4 | 14.2 | ***** | ***** | ***** | ***** |

TABLE 4. *Influence of treatment of oats on development of dextrinogenic enzyme*

| Time of germination (days) | Treatment with carbon disulfide | Treatment with ethylene dichloride | Treatment with ethylene oxide |
|-------------------------------|---------------------------------|------------------------------------|-------------------------------|
| | Dextrinogenic power | Dextrinogenic power | Dextrinogenic power |
| 0 | 243 | 254 | 243 |
| 1 | 706 | 925 | 118 |
| 2 | 2460 | 2430 | 860 |
| 3 | 3400 | 3420 | 940 |
| 4 | 4390 | 4260 | 1382 |
| 5 | 6530 | 6070 | |
| 6 | 7600 | 7830 | 7140 |
| 7 | 6950 | 6950 | 7150 |
| 8 | 4930 | 4100 | 3300 |
| 9 | 7270 | 8325 | 7540 |
| 10 | 4030 | | |

enzymes with comparatively high activity. It is regrettably impossible to classify the action of the chemicals as stimulations or inhibitions because the mold growths, which develop on untreated grain germinated at 20° C. for four days, interfere with the determination of normal amylase activity.

The course of development of saccharogenic and dextrinogenic amylase of oats during germination was tested to determine the number of days required for optimum activity of the enzyme to develop. It was of interest to make a comparison of these tests upon oats treated with carbon disulfide which gave an extract of highest enzyme activity, and with ethylene oxide-carbon dioxide which gave an extract of least enzyme activity. Enzyme tests were made also upon grain treated with ethylene dichloride which showed a low activity for saccharogenic and a comparatively high activity for dextrinogenic enzyme. The results of testing the saccharogenic and dextrinogenic activity of grains treated by these methods and germinated for periods varying from one day to ten days are given in tables 3 and 4.

The oats treated with carbon disulfide show the most rapid formation of saccharogenic enzyme; the enzyme develops more slowly in grain treated with ethylene dichloride, and still more slowly in grains treated with ethylene oxide. It may be noted that, measuring the activity of enzyme by mg. of cuprous oxide per 2 cc. of extract, there is a gradual increase but no maximum. However, when enzyme activity is measured in terms of saccharogenic power, there is a maximum activity and then a drop. This apparent decrease in activity is due partly to an increase in solid matter dissolved or suspended in the extract. The maximum activity of the enzyme varies with the treatment of the grain; it is reached in the carbon disulfide and ethylene dichloride treated grains in five to seven days of germination, and is not reached until the seventh and eighth days of germination of the ethylene oxide treated grain. There is, therefore, either an apparent slowing up of enzyme production in the ethylene oxide treated oats, or a depressing influence of ethylene oxide upon enzyme activity throughout germination.

Table 4 shows that dextrinogenic enzyme is formed rapidly in oats treated with carbon disulfide and ethylene dichloride, while with oats

treated with ethylene oxide, the development of enzyme is greatly retarded until the fourth day of germination when a rapid increase in activity occurs. However, in all three of these seed treatments, the maximum of dextrinogenic activity occurs at six to seven days of germination.

Summarizing the results of these experiments, it may be stated that the maximum activities of saccharogenic and dextrinogenic enzymes are developed in five to seven days of germination when the oats are treated with carbon disulfide or ethylene dichloride, and somewhat later in the ethylene oxide treated oats. It should be noted that Ohlsson and Edfeldt (20) report that the dextrinogenic enzyme does not appear until the ninth day of germination. They made no mention of the necessity of treatment of oats to prevent mold growth during germination. It is evident that the samples of oats here used showed satisfactory development of dextrinogenic amylase within five days of germination under the conditions of these experiments.

The preceding experiments were preliminary to the process of obtaining a purified enzyme preparation from germinated oats. The experiments showed that the highest enzyme activity was obtained with oats treated with carbon disulfide. For these experiments, then, two enzyme extracts were prepared, one from treated grain germinated for three days, and one from treated grain germinated for eight days. There was considerable difference in the enzyme activity of these extracts, as is shown in tables 3 and 4, and it would be of interest to determine whether a more active precipitate could be obtained from the eight-day germinated than from the three-day germinated grain. The method of fractional precipitation with alcohol was applied to the enzyme extracts, in order to bring about directly, a concentration of the enzyme into a precipitate. After adding alcohol to the extracts to make them 50 per cent by volume of alcohol, the precipitate was centrifuged off. The centrifugate from the 50 per cent alcohol precipitate was made up to 65 per cent alcohol and further precipitation occurred. The clear solution from the 65 per cent precipitate was made up to 85 per cent alcohol and a very small amount of precipitate was obtained.

These precipitates were not dried before making tests for enzyme activity as had been done in previous work (7, 17). A dispersion was prepared by suspending a small amount of the wet precipitate in 100 cc. of water, and portions of this solution were used in digestion mixtures. In order to determine the concentration of enzyme in the suspensions, 5 cc. portions were evaporated to dryness and the residues weighed. The determinations for dextrinogenic and saccharogenic power were made on these precipitates, by the methods previously outlined.

The 50 per cent alcohol precipitates had about the same activity from three-day as from eight-day germinated grain. The product from the 65 per cent alcohol precipitation of the extracts showed a higher activity in both cases than from the 50 per cent precipitate, indicating a greater concentration of enzyme in the 65 per cent precipitates. This is in accord with earlier work, and with results obtained in purification of malt amylase by Sherman, Caldwell, and Doebbeling (22). The 85 per cent precipitate showed little enzyme activity.

The 65 per cent precipitate from eight-day germinated grain had a dextrinogenic power of 83,300 and a saccharogenic power of 695. Com-

pared with three-day germinated grain, of which the 65 per cent precipitate showed a saccharogenic power of 77, the precipitate from eight-day germinated grain was nine times more active. The original extract of eight-day germinated grain was considerably more active per milligram of total solid than the extract of three-day germinated grain. Thus, it is evident that the greatest concentration of enzyme in the alcohol precipitate is possible from the original extract which has the highest activity per milligram of solid. Further studies on amylase of oats are in progress.

SUMMARY

1. A study of the amylases of oats has necessarily included a study of the effect of different seed treatments on the development of the enzymes during germination.

2. Fumigation of grain with the heavier-than-air vapors of carbon disulfide, ethylene dichloride, and ethylene oxide-carbon dioxide were found to be effective means of controlling fungus growths.

3. The carbon disulfide treated grain germinated well and showed rapid development of saccharogenic and dextrinogenic enzymes of high activity. The grain that was treated with ethylene oxide-carbon dioxide mixture was slow to germinate and there was no appreciable increase in amylase activity until the fifth day of germination. This treatment affects the saccharogenic and dextrinogenic enzymes alike, and apparently results in a slowing up of enzyme production or permanent injury to the enzyme. Results indicate that the ethylene dichloride treatment had a greater depressing influence upon the saccharogenic enzyme than upon the dextrinogenic enzyme.

4. Enzyme precipitates were made from extracts of carbon disulfide treated oats germinated for three days and for eight days. Fractional precipitation with alcohol produced an enzyme preparation of high activity from the extract of the oats germinated eight days. From this extract, the 65 per cent alcohol precipitate (tested with a dispersion of the undried precipitate in water) showed a saccharogenic power of 695, and a dextrinogenic power of 83,300.

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SOME SPECIFIC TAXONOMIC CHARACTERS OF COMMON LUCILIA LARVAE—CALLIPHORINAE—DIPTERA

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Blowflies of the genus *Lucilia* are commonly known as green-bottle flies. They are scavengers, but are also frequently found infesting living animals. *Lucilia sericata* and *L. cuprina* have been reported as associated with myiasis in livestock in widely scattered regions of the world, but it is probable that other species may be concerned to a greater extent than is usually thought. Their importance as livestock pests appears to vary considerably in different regions, indicating differences in habit. *L. sericata*, on the other hand, has been found to be of much benefit in surgery in hastening the healing of infected wounds of humans. These two species and others may therefore be considered as of distinct importance.

Considerable confusion exists as to the species involved in causing myiasis of different types; and studies, such as the present one, of the immature stages, may tend to clarify the situation. Adequate descriptions of *Lucilia* larvae for use in specific determinations are not available. This is especially true for the first and second instars. The third-instar larva of *L. sericata* has been described by Sinton (6), Banks (2), Patton and Evans (5), and Fuller (3); *L. cuprina* by Patton (5) and Fuller (3); and *L. silvarum* by Banks (2). The first and second instars of *L. sericata* have been described by Tao (7).

The purpose of this paper is to describe certain taxonomic characters of six species of *Lucilia* commonly found in the United States. In each species the three larval instars are described. The species treated are *L. illustris*, *L. sericata*, *L. cuprina*, *L. mexicana*, *L. silvarum*, and *L. caeruleiviridis*. Others are recorded from the United States by Aubertin (1), but are by far less common.

TECHNIQUE

Gravid flies were captured in flytraps or on decomposing rabbits or birds. The species of *Lucilia* are among the first flies attracted to such carcasses. Most of the flies attracted are gravid females ready to oviposit. However, it is expedient to catch only those actually ovipositing, to be certain that they are gravid. These flies were then allowed to oviposit on

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² For synonymy of the various species see Aubertin (1).

liver or lean beef, and the larvae hatching from the eggs were allowed to develop there. Specimens were taken in each stage, killed by dipping in boiling water, and preserved in 75 to 80 per cent alcohol. Some larvae were allowed to develop and the flies retained to check the specific determination.

The clearing and mounting process in the first and second instars consisted in removing the specimens, previously perforated with a fine needle, from the preservative to water for approximately 10 minutes, then boiling them in 5 per cent potassium hydroxide solution for from 5 to 10 minutes. This usually removes or softens the inner portions so that, after the larvae have been placed in water for 15 to 30 minutes, they may readily be squeezed out. The inner tissues may be removed by the boiling solution, but prolonged action of the alkali will often soften or bleach the cephalopharyngeal skeleton, spines, and posterior spiracles to the extent that the form and appearance are changed; therefore, the minimum time in the solution gives the best results. After the inner tissue had been removed the larvae were placed successively in 70, 80, and 95 per cent and absolute alcohol, cleared in xylol, and mounted in balsam on a glass slide. In treating the last-instar larvae the first four or five segments, together with the cephalopharyngeal sclerites, were severed and treated in essentially the same way as described for the first two stages. For a study of the stigmal plates the last segment was severed before clearing and mounting.

CHARACTERS

Constant characters for distinguishing closely related species of blowfly larvae are difficult to establish; for as the larvae grows there are gradual changes in structures within the same stage. This difficulty of distinguishing the larvae is especially true of the species of *Lucilia*, since they comprise a homogeneous group, and most of the structures show enough variation among individual larvae of the same age and species to result in the overlapping of characteristics. Therefore a combination of several characters is usually necessary for correct identification and often it is almost necessary to compare a series of specimens of all ages.

One of the principal characters used by investigators is the structure of the posterior spiracles or stigmal plates. This character is of great value, but it can not be relied upon entirely, since the form of the spiracles may vary, or the peritreme, especially in the region of the button, may be poorly defined in the early period of the last instar, due to lack of pigmentation. The distance between the spiracular plates has frequently been used as a diagnostic character. This is unreliable in all of the species of *Lucilia*, since in these the difference is not marked, for in the early third-instar larvae the plates are always closer together than when the larvae are fully grown. This is true also in *Calliphora coloradensis*, where the measurements of the distance between plates on young third-instar larvae are compared with those between plates on matured larvae from eggs of the same fly, and the distance between plates averages more than twice as much in fully grown larvae as in those that have recently molted. The increase is due to the fact that the distance between the plates increases with the growth of the cuticula while the plates themselves remain of practically the same size. Thus in young third-instar larvae the distance may be less than one-half the width of one plate while in those fully

grown this distance may be equal to or slightly greater than the width of one plate.

The conditions under which larvae are reared may affect the general appearance of the posterior spiracles. At the request of the writer, Mr. S. W. Simmons, of the Bureau of Entomology and Plant Quarantine, kindly consented to rear larvae of *Lucilia sericata* from the same batch of eggs under two different conditions. Some of the hatching larvae were placed on ordinary decaying meat until mature, while the remainder were placed on retarding media for 8 days (approximately 70 per cent of the larvae were in the second instar) and then reared to maturity on ordinary decaying meats. This retarding media was that used in shipping blowfly larvae for wound treatment. The posterior spiracles of the third-instar larvae reared on meat from the time they hatched were similar in form to those of four other series of larvae of this species collected and reared under natural conditions and in different localities. On the other hand, the spiracles of the larvae first fed on retarding media and later on decomposing meat were entirely different and showed very little resemblance to those of the first group. In general the plates were deformed; the slits were short and poorly developed and in practically all of the 110 specimens examined one or more of the slits were almost entirely undeveloped. The peritreme was very irregular and in the region of the button was knob-like in appearance. Such a degree of variation may rarely be found in nature. However, it shows that great variations may exist which should be brought to the attention of those determining closely related species of larvae.

The appearance of one posterior spiracular plate of the last-instar larva as shown in the figures (plates I and II) is representative of the species and is based on examinations of mature larvae from two or more series. Although there are some variations among individual larvae reared under identical conditions, one familiar with the variations by examining large numbers of larvae of different ages can usually recognize certain characteristics typical of the species.

In the first and second instars the posterior spiracles are similar in all of the species and are of no great value as distinguishing characteristics.

The size of the cephalopharyngeal sclerites has only a limited value as a specific character. In order to determine the possible variation in the size of this structure, larvae from the eggs of one gravid female of *Lucilia sericata* were taken; one-half were placed on an abundant supply of moist meat in an open container, and the other half placed, under less favorable conditions, on a small quantity of almost dry meat in a container with a limited amount of air. Those in the first lot developed into large larvae, while those of the second made little progress and only a small percentage pupated and produced flies. The length of 25 cephalopharyngeal sclerites from the first group ranged from 1.246 mm. to 1.432 mm. with an average length of 1.306 mm., whereas a like number from the second ranged from 1.060 to 1.173 mm. with an average of 1.110 mm.

The cephalopharyngeal mechanism in all of the species and in each instar is very similar, and, as with the posterior spiracles of the third-instar larvae, some variations in structure are noted. However, specific differences are apparent in most of the species. No attempt has been made to give detailed descriptions of the sclerites of the cephalopharyngeal

mechanism. Differences between species are more readily noted by comparing the figures. The cephalopharyngeal mechanism in the first instar is not figured in three species, but in the descriptions differences are pointed out between the structures of those figured and those not figured.

All of the larvae of *Lucilia* are provided with a large number of small, brownish, recurved spines. These spines occur in irregular, closely approximated rows to form bands of spines on the anterior and posterior borders of the segments. The number of segments having a complete band of spines encircling the body on either or both the anterior and posterior borders is a good supporting character although rather variable. The distribution of spines may be different in the three stages of the same species.

On the posterior border of the twelfth or last apparent segment are a number of rather prominent fleshy processes or tubercles (plate II, fig. 13). There are generally six on each of the upper and lower borders in the second and third instar larvae, but in the first instar all of the tubercles are seldom apparent. In the third-instar larvae the relative size and position of the tubercles outlining the stigmal field furnish one of the most useful characters in separating the species. The tubercles are of less diagnostic value in the first and second instar larvae than in the third but are of considerable value, especially in the second. A prominent tubercle is situated on either side and near the end of the anal protuberance (AT, fig. 13, plate II). The size of these tubercles as compared to those bordering the stigmal field is of occasional value.

On each side and near the base of the second apparent segment are located the small branched anterior spiracles. The number of branches in the anterior spiracles frequently has been used as a diagnostic character. A study of a large number of specimens of several species shows that the variation in number of branches is so great that this character should not be considered as a reliable one, and can only be considered a supporting character for some of the species. The number of branches of the anterior spiracles seems to vary in *Lucilia* larvae found in different geographical regions. Patton (4) reports 6 to 8 branches (generally 8) in third-instar larvae of *Lucilia cuprina* (*argyricephala*) from India. Fuller (3) reports 7 to 8 branches in the same species from Australia. The writer examined two series of larvae of this species, one from Dallas, Texas, and the other from Port Lavaca, Texas, and in each series the number of branches ranged from 5 to 6. In the third-instar larvae of *Lucilia sericata*, Patton and Evans (5) report 10 to 11 branches and Fuller (3) 10 branches. The variation in the number of branches of the anterior spiracle of this species in four series of larvae, one of which was obtained from Dallas, Texas, two from Ames, Iowa, and one from Washington, D. C. (surgical maggot culture), was 7 to 10, 7 to 9, and 6 to 9, and 5 to 9 branches, respectively. The corresponding average number of branches for each of the above series was 8.6, 7.9 and 7.5, and 6.7.

The data above show that the number of spiracular branches in different series of this species may vary to such an extent that this character is of little specific value.

A general description of the larvae in each instar is given and followed by specific descriptions for each species and stage. The third instar is first described, followed by descriptions of the second and first, respectively.

GENERIC DESCRIPTION

Third instar.—Full-grown maggots 12-15 mm. in length and creamy white to light yellowish in color. Posterior half cylindrical, anterior half tapering anteriorly to the cephalic segment, terminating in two prominent, fleshy anterior maxillary lobes each bearing a pair of sensory papillae (photo-receptive organs). The mouth is situated below on the cephalic or first apparent segment, which bears on each side a semi-circular striated area, the stomal disc, with striations converging more or less to the oral groove. At the posterior border of the cephalic segment is a broad band of small, brownish, recurved spines. The band is widest on the ventral side. At the anterior border of the second apparent segment a narrow band of spines is present ventrally and laterally, but absent dorsally. Near the base and on each side of segment 2, is situated a fan-shaped structure, the anterior spiracle; each of these spiracles bears a number of lobes or branches, the number ranging from 5 to 10 or more. At the anterior border of segments 3-5 the bands of spines are approximately the same width throughout, while on segments 6-12 the ventral spinose area is broader and divided transversely by a narrow spineless area. The anterior border of segments 2-7 is always completely encircled with a band of posteriorly-directed spines; the band may or may not be complete on segments 8 and 9, depending on the species; the band is absent dorsally at the anterior border of segments 11 and 12. One or more of the last segments is usually provided with anteriorly directed spines at the posterior margin of the segments. On the lateral side of segments 5-12 is located a small swollen fusiform area bearing a number of small spines. The last apparent segment is shorter than the others, obliquely truncate, and slightly depressed behind (fig. 13, plate II). This segment bears the two posterior spiracular plates, situated in the depressed area or stigmal field. Each of the borders, upper and lower, of the stigmal field has three pairs of conical fleshy processes or tubercles; the median pair on the lower border is more remote from the margin and smaller than the others. A small, inconspicuous pair of tubercles is situated in the lower portion of the stigmal field above the lower border. A conspicuous tubercle is situated at the end on each side of the anal protuberance (AP, fig. 13, plate II).

The posterior spiracles are rounded to pear-shaped plates; three long straight to slightly bent slits are in each plate, directed downward and inward. Surrounding the slits is a pigmented ring or peritreme. A small oval to circular clear space is located along the side of each slit, from which protrudes a number of clear ray-like structures; a similar structure is located between and near the upper end of the inner and middle slits (R, fig. 2, plate I).

The cephalopharyngeal mechanism is located in the first four or five segments and is made up of a number of loosely connected sclerites. The pair of oral hooks (OH, fig. 1, plate I) is situated at the anterior end and consists of two slightly curved and tapering hooks, each joined to a heavy, broad basal portion; below the basal portion on each side is a small dental sclerite (D). The hypostomal sclerite (H), an H-shaped structure when viewed from below or above, is located between the oral hooks and the large paired pharyngeal sclerites (PHS). The pharyngeal sclerites are joined dorso-anteriorly by a dorsal arch (DA). The dorso-posterior and

ventro-posterior portions of the pharyngeal sclerites form the dorsal (DC) and ventral cornuae (VC), respectively. On each side near the base of the anterior portion of the pharyngeal sclerite is a small rod-shaped structure, the parastomal sclerite (PS), extending anteriorly above the hypostomal sclerite.

Species of *Lucilia* in the third instar may be readily distinguished from those of *Calliphora* and *Cynomyia* by the absence of a small rod-like structure below and between the tips of the oral hooks, and from *Phormia*, *Cochliomyia*, *Chrysomyia*, and *Sarcophaga* by the presence of a complete peritreme around each posterior spiracle.

Second Instar.—General features similar to those of the third instar but smaller. Second-instar larvae may be distinguished from the third, externally, by the posterior spiracles; there are, as with other blowflies, only two slits to the spiracles, and these are surrounded by an incomplete ring or peritreme. The anterior spiracles are similar to those of the third-instar larvae but smaller and usually with a longer and more slender stigmatic chamber. The relative position and size of the tubercles on the last segment are in general similar to those of the third instar.

The cephalopharyngeal mechanism is shown in figures 14-19. The bases of the oral hooks are longer in proportion to the width than they are in the third instar and not so strongly developed; the hooks proper are produced upward and curve sharply downward near the enlarged tip.

The oral hooks of the second instar of *Calliphora* and *Cynomyia* are much broader in proportion to the length, and in *Phormia*, *Cochliomyia*, and *Sarcophaga* the hooks gradually taper from the basal portion to the tip. Although there are other differences these will readily distinguish the larvae of *Lucilia* from those of the genera mentioned.

First instar.—Newly hatched larvae are from 2.0 to 2.5 mm. in length. The general form is the same as in the third and second instars. The spines are small and almost colorless to dark brown in color; the distribution of the spines in general is similar to that of the third and second instar. The posterior end has three pairs of very small tubercles on the upper and three pairs on the lower border of the stigmal field; the median pair on the upper border and the inner pair on the lower border are usually poorly defined.

The two posterior spiracles are small heart-shaped structures, each one having two apertures and not surrounded by a peritreme. The anterior spiracles are not apparent (metapneustic).

The cephalopharyngeal sclerites are weakly developed and the degree of pigmentation varies considerably with the age of the larvae; in newly hatched larvae the sclerites are yellowish in color and shortly before the larvae molt to the second instar they are almost black. Oral hooks, composed of a pair of rod-like structures, have a number of small denticles at the anterior end which are loosely joined to form the hooks; these denticles are directed downward. A small, usually bluntly pointed hatching spine (HS, fig. 20, plate II) is located above and posterior to the oral hooks and above the hypostomal sclerites. A pair of long, slender, rod-like structures, the parastomal sclerites (PS, fig. 20, plate II) are attached to the pharyngeal sclerites, extend anteriorly above the hypostomal sclerites, and join with the posterior basal portion of the hatching spine. The pharyngeal sclerites are rather short and broad; the dorsal

and ventral cornua are very nearly equal in length and widely separated (figs. 20-22, plate II).

DESCRIPTION OF SPECIES

Lucilia illustris (Meigen)³

Third instar.—General features as described in generic description. Belts of spines encircle the body at the anterior margins of segments 2-9; on segment 10 the band of spines is generally incomplete dorsally for a short space, while on segment 11 spines above the venter are restricted to one or two irregular lateral rows. Posterior border of segment 11 with 5-6 irregular dorsal rows of spines; segment 10 usually with 1-2 irregular dorsal rows, but these may be absent for a short space; dorsal spines generally absent on segment 9 and those anterior to it.

Conical tubercles or fleshy protuberances outlining the stigmal field rather large and prominent; the inner and outer pairs on upper border very nearly equal and about twice as large as the median, the two inner tubercles separated by a distance greater than the distance between the inner and median on each side; approximately equal to the distance between inner and outer on each side (similar to *L. mexicana*, fig. 13, plate II). Outer and median pairs on lower border large, approximately equal in size, and much larger than the inner pair. The inner pair on lower lip smaller than the outer pair on upper border. Tubercles on anal protuberance slightly shorter and less pointed than the two outer pairs on lower border of stigmal field. Segment 12 generally smooth to finely scabrous on dorsal surface.

Posterior spiracles comparatively large, heavily pigmented, and with usual form as shown in figure 2, plate I. The peritreme (P) is prominent, with a very well developed inward projection between the outer and middle slits.

Cephalopharyngeal apparatus large and heavily pigmented. The dorsal cornu broad and widely separated from the ventral cornu. At the posterior end and below the ventral cornu is a pigmented porous area (PO, fig. 1, plate I), possibly a more heavily pigmented portion of the pharynx, which is not prominent in other species of *Lucilia* described here. The oral hooks pointed slightly upward and curved downward.

The anterior spiracles with comparatively large prominent branches. The external portion equal to or only slightly longer than stigmatic chamber (pigmented portion of the trunk). Anterior spiracles generally with 6-8 branches. The number in 80 spiracles ranged from 5-9 in ratio 5-23-20-22-2, respectively; number of branches rather uniform in three series.

Three series of larvae examined; one from Galesburg, Ill., and two from Ames, Iowa.

Second instar.—The anterior border of segments 2-9 with a complete band of spines. Dorsal spines absent on segment 10, but 1 or 2 rows may extend well up to the dorso-lateral surface. Posterior border of segments 9-12 with a complete band of spines; on segment 9 they are reduced to one or two irregular rows and on segment 8 are restricted to the ventral and lateral surfaces.

³ Formerly incorrectly known as *L. caesar*, a species found in Europe, Morocco, China, and Japan. Aubertin (1).

Tubercles on upper border of last segment broadly rounded and in relative size similar to those of the third instar. The inner pair on lower border approximately one-third as large as the median and outer pair. The distance between the inner tubercles on upper border as great or greater than distance from inner to outer.

Cephalopharyngeal mechanism with dorsal cornu of almost same width to the posterior end, where it sharply tapers to a point. The dorsal posterior projection of the basal portion of the oral hooks broad and bluntly rounded (fig. 14, plate II).

Anterior spiracles with 6-8 branches.

First instar.—Distribution of spines rather difficult to determine owing to lack of pigmentation on some of the segments. Anterior border of segments 2-9 with a complete band of spines. On segment 8 the spines are lighter in color on lateral and dorsal surfaces, on 9 they are even lighter in color, being almost colorless; number of rows of spines reduced to one or two on segment 9; on segment 10 several rows of light-colored spines are present laterally and may extend to the dorso-lateral surface but do not join to form a complete band. Posterior border of segment 11 with two to three rows of rather dark spines dorsally, while on segment 10 the spines are narrowed to one or two rows of lighter spines; the dorsal surface of segment 9 may or may not be provided with one to two irregular rows of almost colorless spines at the posterior border. Border of posterior cavity with small pigmented hair-like spines.

Tubercles on segment 12 with the inner pair on upper border separated by a distance approximately equal to the distance from the inner to the outer on either side; the median pair on upper border and inner pair on lower border inconspicuous.

Cephalopharyngeal mechanism as shown in figure 20, plate II. The pharyngeal sclerite is comparatively strong and heavily pigmented; dorsal and ventral cornu approximately equal in length and width; anterior projection of pharyngeal sclerite rather short and joined by a narrow dorsal arch. Hatching spine well developed.

Lucilia sericata (Meigen)

Third instar.—Distribution of spines rather variable; anterior margin of segments 2-8 provided with complete encircling band of spines; on segment 9 the spines may also be present on the dorsal surface to form a complete band, but more usually are absent for a short space; segment 10 may or may not be provided with 1 to 2 irregular broken rows on the lateral surface. The distribution of spines appears to be more uniform in the same series; in one series a large majority of the larvae had a complete band of spines on segment 9, while in another series very few of the larvae were provided with a complete band of spines on this segment. The posterior border of segment 11 has about four irregular rows of spines, while on segment 10 they are absent on the dorsum and usually restricted to the ventral and ventro-lateral surfaces. The twelfth segment is smooth on the dorsal surface.

Tubercles outlining stigmal field and on anal protuberance large; those on the latter much larger than those on former. Comparative size of tubercles on border of stigmal field as in *L. illustris*. The distance between the inner tubercles on upper border approximately equal to the dis-

tance between the inner and median, and never separated by a distance equal to that between the inner and outer on each side.

Posterior spiracles with usual form as indicated in figure 4, plate I; peritreme narrow, yellowish, never black or dark-brown; inner projection of peritreme, if present, usually faint and poorly developed but often absent entirely.

The cephalopharyngeal mechanism as shown in fig. 3, plate I. The dorsal cornu rather widely separated from the ventral.

Anterior spiracles with a variable number of branches (see discussion of anterior spiracles), more often with 7-8 branches. The branches are rather prominent and the portion external to the cuticle is approximately equal in length to the stigmatic chamber.

Descriptions based on five series of larvae, one each from Dallas and Menard, Texas, and Washington, D. C. (surgical maggots), and two from Ames, Iowa.

Second instar.—Anterior border of segments 2-7 provided with a complete band of spines; segment 8 usually with a narrow dorsal spineless area, but spines may also be present to form a complete band on this segment. On segment 9 the spines extend from the ventral to the dorso-lateral surfaces but are absent on the dorsum; generally restricted to ventral surface on segment 10. Posterior border of segments 11 and 12 provided with a complete band of spines, narrowed to 1 or 2 irregular rows or sometimes absent entirely on the dorsal surface of segment 10.

The distance between the inner pair of tubercles on upper lip approximately equal to the distance between inner and median pairs and never equal to the distance between inner and outer pairs. This is the most reliable character for distinguishing this species from the other species of *Lucilia* in this stage.

Cephalopharyngeal apparatus very similar to that of *L. illustris* (fig. 15, plate II).

Anterior spiracles more often with 7-9 branches.

First instar.—Spines lightly pigmented. Segments 2-7 completely encircled with spines at the anterior border; on segment 8 they are absent on the dorsal surface but may extend well up. Posterior border of segments devoid of spines on the dorsal surface, except on segment 12, which is provided with long, almost colorless spines on the border of the posterior cavity.

Size of tubercles on segment 12 similar to those of *L. illustris*, but the inner pair on the upper border separated by a distance much less than the distance from inner to outer.

Cephalopharyngeal apparatus similar to that of *L. illustris*, except that the dorso-anterior projection of the pharyngeal sclerite is longer and the ventral cornu are usually longer than the dorsal.

Lucilia cuprina (Wiedemann)

Third instar.—Spines not prominent, lightly pigmented. Anterior border of segments 2-8 completely encircled with spines; segment 9 generally with one or two irregular broken dorsal rows, but these may be absent for a short space. Spines restricted to ventral and lateral surfaces on segment 10. Posterior border of segment 11 with three to four rows of dorsal spines; dorsal spines absent on segment 10. The distribution of spines as given is similar to that of *L. sericata*, but the spines are smaller

and lighter in color and seemingly less variable in distribution. Segment 12 smooth on dorsal surface, except on posterior margin.

The tubercles around the stigmal field are not nearly so prominent and much smaller than in the two preceding species. The outer pair on upper border distinctly larger than inner and more than twice as large as the median; the inner pair on lower border less than one-half as large as the other two pairs. Relative position of tubercles similar to that in *L. illustris*. Tubercles on anal protuberance about twice as large as the larger ones on the lower border of the stigmal field.

Posterior spiracles small and broad in comparison to the length, more often broader than long. Slits short. Peritreme without inner projections (fig. 6, plate I).

Cephalopharyngeal apparatus small, approximately 1 mm. in length. This is considerably smaller than in other species. The pharyngeal sclerites are comparatively short and broad (fig. 5, plate I).

Anterior spiracles with 5-6 branches; the ratio in 64 spiracles, 40-24, respectively; the number similar in two series of larvae. Portion of anterior spiracle external to the cuticle approximately one-half as long as the internal pigmented trunk.

Descriptions based on two series of larvae, one each from Port Lavaca and Dallas, Texas.

Second instar.—The spines are small and light brown to almost colorless. Segments 2-7 with complete band of spines at anterior border; on segment 8 the spines are usually absent or very small and lightly pigmented on the dorsal surface. Posterior border of segment 11 with several rows of dorsal spines; absent on dorsum of segment 10.

Tubercles on border of stigmal field small; relative position as in the third instar.

The cephalopharyngeal sclerites are more distinct in this species and may easily be distinguished from those of other species. The most striking difference may be noted in the appearance of the oral hooks (fig. 16, plate II). The entire length from the tip of the oral hooks to the tip of dorsal cornu 500-550 microns—while in all other species the length is at least 600 and may be greater than 700 microns.

Anterior spiracles with 4-6 widely separated branches; more often 5.

First instar.—Spines lightly pigmented, more so than in *L. sericata*; they are almost without pigment and their distribution is difficult to determine. Anterior border of segments 2-7 with complete band of spines. Posterior border of segment 11 with two to three dorsal rows of highly pigmented spines; apparently absent on dorsal surface of segment 10 and those anterior to it.

Tubercles on segment 12 small; relative position as in the second and third instars.

Cephalopharyngeal apparatus very small and weakly developed (fig. 21, plate II). Oral hooks not prominent; hatching spine short. Pharyngeal sclerites narrow; ventral cornu narrow and tapering to a sharp point. Dorso-anterior projection of pharyngeal sclerites long, approximately two-thirds as long as the dorsal cornu.

Lucilia silvarum (Meigen)

Third instar.—Spines encircling the body at the anterior border of segments 2-9. On segment 10 several rows of spines extending almost to

the dorsal surface, and on segment 11 two or three irregular broken rows of spines present laterally. Posterior border of segments 9-12 with a complete band of spines; segment 11 with 6-8 irregular rows dorsally and about 4 and 3 rows, respectively, on segments 10 and 9. Segment 8 may have a single irregular dorsal row, but this is usually absent for a short space. Segment 12 is very finely scabrous over the entire dorsal surface; in other species this area is smooth except on *L. illustris*, which, however, usually has a small, irregular, smooth area.

As with *L. cuprina* the tubercles outlining the stigmal field are smaller and less conspicuous than in the other species of *Lucilia*; the median pair on the upper and the inner pair on the lower border of the stigmal field less than one-half as large as the others. Tubercles on anal protuberance low, broadly rounded at the tip.

Posterior spiracles rather small, of the general form shown in figure 8, plate I. Peritreme wide and irregular; inner projections usually absent, but there may be a short pointed pigmented projection between the middle and lower slits.

Cephalopharyngeal apparatus with the dorsal cornu close to the ventral (fig. 7, plate I).

Anterior spiracles with small, short branches, the part external to the cuticle approximately one-half the length of the spiracular chamber. The number of branches in 52 spiracles from two series of larvae ranged from 5-7 in ratio of 8-23-21, respectively; rather uniform in the two series.

Larvae collected at Ames, Iowa.

Second instar.—Segments 2-9 provided with a band of spines encircling the body at the anterior border; present on lateral surface of segment 10, but absent on dorsal surface. Posterior border of segments 9-12 with a complete band; narrowed to 1 or 2 irregular dorsal rows on segment 9.

Relative position of tubercles on stigmal field as in the third instar; broad at base and somewhat pointed at tip.

Cephalopharyngeal apparatus as in figure 17, plate II. The pharyngeal sclerite is rather lightly pigmented; the oral hooks are rather narrow and, with the exception of *L. cuprina*, the dorsal posterior prolongation of the basal portion is narrower than in the other species. The shape of the dorsal cornu is about intermediate between that of *L. illustris* and that of *L. caeruleiviridis*.

Anterior spiracles with 5-7 branches, more often with 6.

First instar.—Spines distinct, heavily pigmented. Complete band of spines at anterior border extending to segment 9. On segment 10 the spines are usually absent dorsally but extend well up to the dorso-lateral surface. Posterior border of segments 9-12 completely encircled with spines; on segment 9 the band is narrowed to 1 or 2 dorsal rows; restricted to lateral and ventral surfaces on segment 8. The border of the stigmal field is provided with brownish, long, hair-like spines.

Tubercles of the last segment as in *L. illustris*.

Cephalopharyngeal apparatus as shown in figure 22, plate II. The parts are rather well developed. The principal distinguishing feature is the broad dorsal pharyngeal arch, this being much broader than in the other species.

Lucilia caeruleiviridis (Macquart)

(australis Townsend)

Third instar.—Anterior margin of segments 2-9 with a complete band of spines; on segment 10 the spines extend well up to the dorso-lateral surface but do not join. Spines rather variable at posterior border of segments; segments 10-12 with spines dorsally; six to eight rows usually present on dorsal surface of segment 11. On segment 9 the spines may join by a single irregular dorsal row to form a complete band, but may be absent on dorsum by a wide margin. Segment 12 generally smooth on dorsal surface.

Tubercles outlining the stigmal field very large and prominent, the outer and inner pairs on upper border approximately equal and less than twice as large as the median. The distance between the inner pair at the tips greater than the distance between the inner and intermediate, but not so great as the distance from the inner to the outer pair. The two outer pairs on the lower border larger than those on anal protuberances and usually larger than the larger pair on upper border of stigmal field.

Button area of spiracular plates rather well developed, with a cylindrical pigmented inward projection below the button which is visible only when mounted plates are examined. Peritreme well developed; inward projection of the peritreme between outer and middle slit prominent. Spiracles in general heavily pigmented as in *L. illustris*, especially at the border and around the slits. However, the degree of pigmentation, as with other species, varies considerably with the age of the larvae.

The cephalopharyngeal apparatus (fig. 9, plate I) similar to that of *L. silvarum* and *L. mexicana*. The ventral cornu is produced posteriorly beyond the hump to a greater extent than in the two species mentioned. Oral hooks curved slightly upward and downward.

Anterior spiracles with the number of branches variable; one series of 62 spiracles gave ranges from 6 to 10 in ratio 4-16-21-18-3, while the number of branches in 24 spiracles in another series ranged from 7 to 10 in ratio 1-5-8-9. Thus in one series 7, 8, and 9 branches predominated and there were only a small number with 6 and 10 spiracles, while in the other 8, 9, and 10 branches predominated and there were only a small number with 7 branches. The branches are small and short and the length of the external portion is approximately equal to that of the spiracular chamber.

Two series of larvae examined collected at Ames, Iowa.

Second instar.—Distribution of spines the same as in *L. silvarum*.

Tubercles on border of stigmal field rather large; the median pair on upper border not much smaller than the outer. On the lower border the inner pair more than one-half as large as the outer and median. Distance between inner tubercles on upper border distinctly less than the distance between the inner and outer, but greater than the distance from the inner to the median on either side.

Cephalopharyngeal apparatus as in figure 18, plate II. The dorsal cornu tapers to the posterior end. The oral hooks are rather short and broad.

Anterior spiracles with 7-9 branches, more often with 9.

First instar.—The distribution and general appearance of spines very similar to *L. silvarum*.

This species may be distinguished by the cephalopharyngeal apparatus. The general appearance is similar to *L. silvarum* except that the dorsal arch is narrow, resembling that of *L. illustris*.

Lucilia mexicana (Macquart)

(*unicolor* Townsend)

Third instar.—Distribution of spines variable. Anterior margin of segments 2-8 generally completely encircled with spines; in some specimens, however, the complete band may extend to segment 9, while in others it may extend only to segment 7. Posterior margin of segment 11 generally provided with three to four dorsal rows of spines, but spines may be absent at the posterior margin of all but segment 12.

Tubercles outlining the stigmal field rather prominent (fig. 13, plate II); the inner pair on upper border equal to or only slightly larger than the other two pairs, which are very nearly equal in size. On the lower border the outer and intermediate pairs large; the inner pair comparatively large, approximately equal to the outer tubercles on the upper border, whereas in other species the inner tubercles on the lower border are always distinctly smaller. The relative position of the tubercles as in figure 13.

Posterior spiracles as shown in figure 12, plate II. Peritreme narrow, not heavily pigmented. A rather narrow and long inward projection of the peritreme between inner and lower spiracular slits.

Pharyngeal sclerites (fig. 11, plate II) similar to those of *L. caeruleiviridis* but with shorter ventral cornu.

Anterior spiracles in one series with 5-8 branches in ratio 1-7-12-4; in another, 6-11, in ratio 3-28-33-15-4-1. Size of lobes and form of spiracles as in *L. sericata*.

Descriptions based on three series of specimens collected, one each, from Port Lavaca, Dallas, and Menard, Texas.

Second instar.—Spines forming a complete band at anterior border of segments 2-8 and extending almost to the dorsal surface on segment 9. Posterior border of segments 11 and 12 with complete band of spines; on segment 10 the spines usually forming a complete band but sometimes restricted to the ventral, lateral, and dorso-lateral surfaces.

Relative distribution of tubercles situated on last segment as in the third instar. Tubercles broadly rounded at tip, the median pair on upper border and inner pair on lower border only slightly smaller than the others.

Cephalopharyngeal apparatus similar to that of *L. caeruleiviridis* but with more slender oral hooks and the basal portion with a longer dorso-posterior projection.

Anterior spiracles with 6-8 branches.

First instar.—Spines heavily pigmented as in the two preceding species. A complete band of spines at the anterior border extending to segment 8. Posterior border of segments 10 and 11 provided with spines on dorsal surface; on segment 9 the spines extending well up to the dorsal surface but both the anterior and posterior margins with a narrow dorsal spineless area.

Tubercles on last segment and cephalopharyngeal skeleton similar to those of *L. illustris*.

Key to third-instar larvae of Lucilia

1. Pharynx with a prominent pigmented area below the posterior extremity of ventral cornu (PO, fig. 1, plate I) *Lucilia illustris* (Meig.)
 Pharynx without this pigmented area..... 2
2. Inner tubercles on upper border of stigmal field widely separated; separated by a distance approximately equal to the distance from the inner to the outer on either side..... 3
 Inner tubercles on upper border of stigmal field not widely separated; separated by a distance equal to or only slightly greater than distance from inner to median on either side..... *Lucilia sericata* (Meig.)
3. Median pair of tubercles on upper border of stigmal field distinctly smaller than the outer pair..... 4
 Median pair of tubercles on upper border of stigmal field equal to or only slightly smaller than the outer pair..... *Lucilia mexicana* (Macq.)
4. Tubercles bordering the stigmal field not prominent; inward projection of peritreme between outer and middle slits absent or at least not prominent; anterior spiracles generally with 5-7 branches..... 5
 Tubercles bordering the stigmal field large, resembling those of *L. mexicana* (fig. 13, plate II) in prominence; spiracular plates large, dark, and with prominent inward projection between outer and middle slits, anterior spiracles generally with 7-10 branches.....
 *Lucilia caeruleiviridis* (Macq.)
5. Spines light-brown; posterior margin of segments 9 and 10 without dorsal spines; 3-4 dorsal rows on posterior margin of segment 11 *Lucilia cuprina* (Wied.)
 Spines dark-brown; posterior margin of segments 9-12 completely encircled with spines; 6-8 dorsal rows on posterior margin of segment 11 *Lucilia silvarum* (Meig.)

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EXPLANATION OF PLATES

PLATE I

Fig. 1 and 2. *Lucilia illustris* Meigen

- (1) Lateral view of cephalopharyngeal mechanism, third-instar larva; OH, oral hook; D, dental sclerite; H, hypostomal sclerite; PS, parastomal sclerite; DA, dorsal arch; PHS, pharyngeal sclerite; PH, pharynx; DC, dorsal cornu; VC, ventral cornu; PO, pigmented portion of pharynx. $\times 52$.
- (2) Right spiracular plate. P, peritreme; B, button; R, sun-ray structures. $\times 132$.

Fig. 3 and 4. *Lucilia sericata* Meigen. Same as figures 1 and 2.

Fig. 5 and 6. *Lucilia cuprina* Wiedemann. Same as figures 1 and 2.

Fig. 7 and 8. *Lucilia silvarum* Meigen. Same as figures 1 and 2.

Fig. 9 and 10. *Lucilia caeruleiviridis* Macquart. Same as figures 1 and 2.

PLATE I

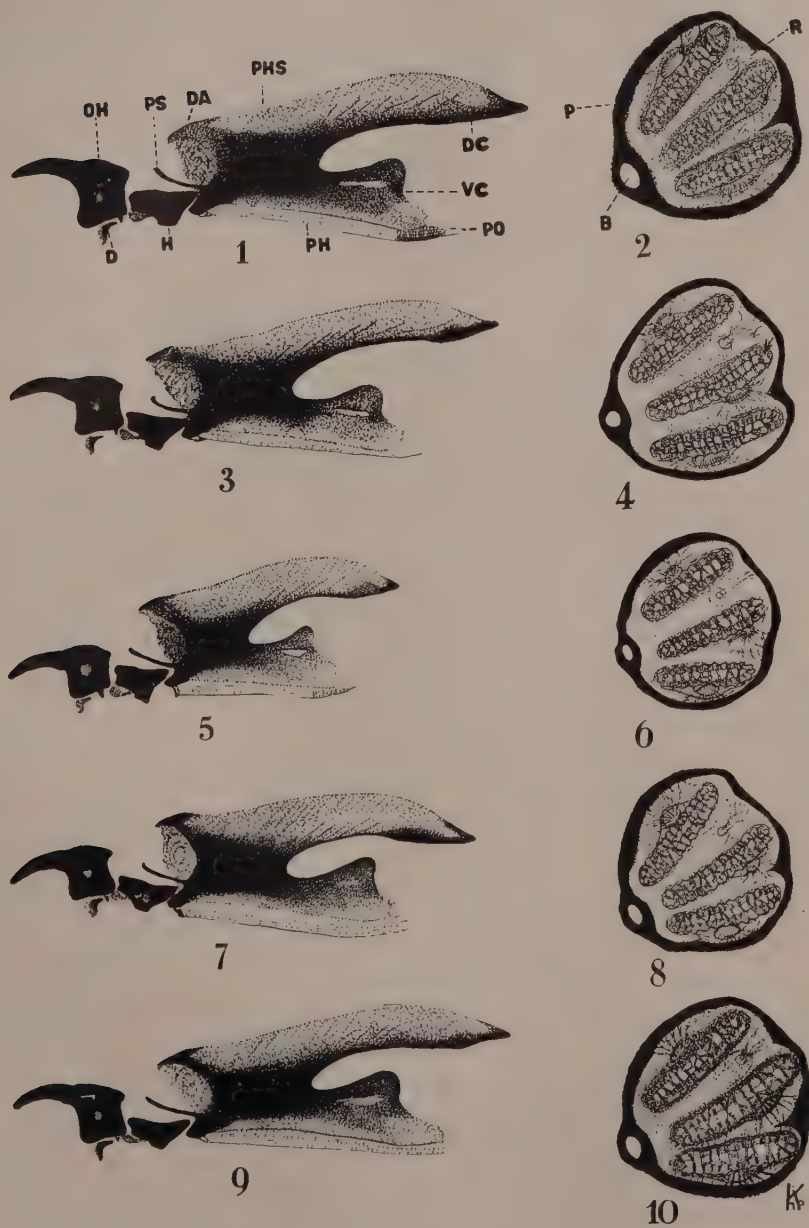
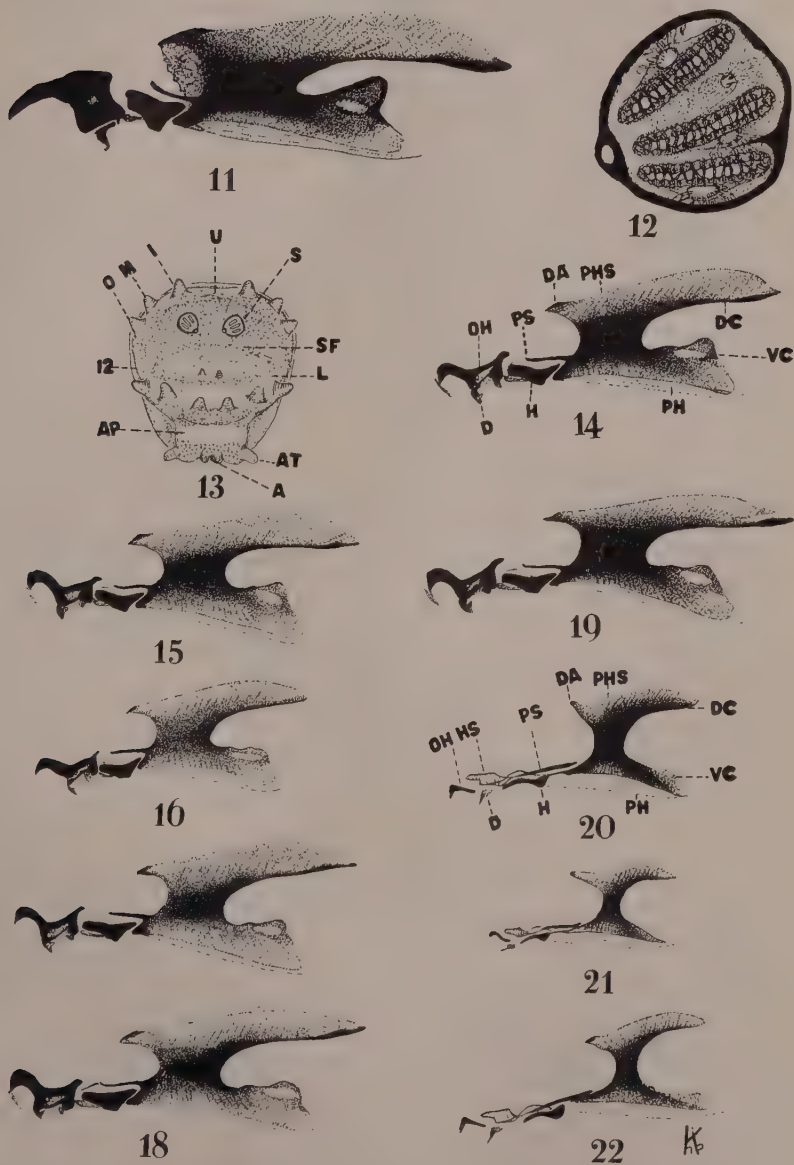


PLATE II

- Fig. 11 and 12. *Lucilia mexicana* Macquart. Same as figures 1 and 2, plate I.
- Fig. 13. *Lucilia mexicana* Macquart. Last apparent segment of third-instar larva showing position of spiracular plates and structure and position of tubercles outlining the stigmal field or posterior cavity; O, M, and I, outer, median, and inner tubercles, respectively; U, upper border of stigmal fields; S, spiracular plate; SF, stigmal field; L, lower border of stigmal field; AT, anal tubercle; A, anal opening; AP, anal protuberance; 12, segment 12.
- Fig. 14. *Lucilia illustris* Meigen. Lateral view of cephalopharyngeal mechanism; second-instar larva. $\times 72$. Lettering as in figure 1, plate I.
- Fig. 15. *Lucilia sericata* Meigen. Same as figure 14.
- Fig. 16. *Lucilia cuprina* Wiedemann. Same as figure 14.
- Fig. 17. *Lucilia silvarum* Meigen. Same as figure 14.
- Fig. 18. *Lucilia caeruleiviridis* Macquart. Same as figure 14.
- Fig. 19. *Lucilia mexicana* Macquart. Same as figure 14.
- Fig. 20. *Lucilia illustris* Meigen. Lateral view of cephalopharyngeal mechanism; first-instar larva. $\times 120$. HS, hatching spine; other lettering as in figure 1, plate I.
- Fig. 21. *Lucilia cuprina* Wiedemann. Same as figure 20.
- Fig. 22. *Lucilia silvarum* Meigen. Same as figure 20.

PLATE II



A MACRO-RESPIROMETER FOR THE STUDY OF AEROBIC BACTERIAL DISSIMILATION¹

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Manometric methods for the study of respiration of isolated tissues and of microorganisms have been in wide use during the last few years. Dixon (1934) has summarized the principles and techniques of manometric determinations. The methods developed usually have been adopted to micro-analysis and have involved the use of a small quantity of medium, generally less than 10 ml. The gaseous compounds ordinarily have been the only products determined as the small quantity of substrate precludes the determination of other products. In the present article a macro-respirometer is described which permits determinations of O₂-uptake as well as all products of dissimilation with macro-methods.

APPARATUS

The constant-pressure type of respirometer has been used because its theory is simple and flask constants are not employed. Absence of constants allows changes of the flask to be made with no redetermination of constants.

A complete unit of the constant-pressure manometer is shown in Fig. 1; a series of these units arranged on a mechanical shaker is shown in Plate I. The dissimilation occurs in L (fig. 1), which is a 3-liter Fernbach flask containing the alkali cup A with rolls of filter paper B, and the container C by which the cell suspension is added to start the dissimilation. Flask L is connected by rubber tubing to the graduated gas burette E. G is a compensation flask containing a volume of uninoculated medium equivalent to that in L. The two flasks are connected through a manometer tube F which shows differences in pressure. Tube D is used in removing samples and H, I, J, and K are screw clamps. The apparatus has been used by the authors in a constant temperature incubator room. Only one compensation flask is necessary for a group of reaction vessels and several may be connected to the manometer F through branched glass tubing and balanced separately against G by opening the screw clamp J.

Operation of the apparatus in taking gas readings is simple. At the beginning of the experiment all screw clamps are opened. After the flasks and medium have attained constant temperature the clamps are closed and the barometric pressure, temperature, and reading of gas burette are noted. To make subsequent readings J is opened and the liquid in F is adjusted to the same level in the two arms by raising or lowering E and the gas burette is read. Difference between readings gives the change in the volume during the period and at the temperature and barometric

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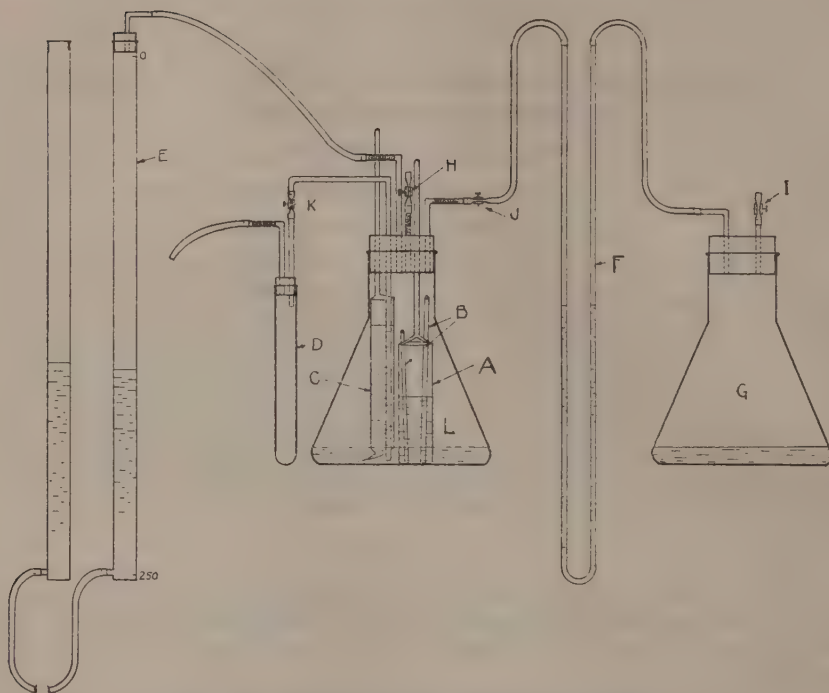


Fig. 1. Diagram of unit of macro-respirometer.

pressure recorded at the time the compensation flask was closed. This volume must then be reduced to ml. of dry gas at normal temperature and pressure.

If the compensation vessel and the manometer were not used, and the readings made by adjusting the liquid in the gas burette then any slight change in temperature or barometric pressure would cause considerable effect on the readings. The flask G serves as a control with which the reaction flask may be compared, so that the apparatus is no longer affected by variations of temperature or barometric pressure. This is true since G is a closed system at constant volume. Any rise or fall of temperature increases or decreases the pressure in G and the volume of gas in flask L. This increase or decrease in volume of L is compensated for when the pressure is raised or lowered to that of G.

Use of the Fernbach flask permits introduction and removal of containers C and A; its wide bottom allows use of a considerable quantity of liquid and gives a thin layer suitable for aerobic experiments. The large capacity permits the consumption or production of considerable quantities of gas without changing the composition in the flask excessively. Alkali cup A may be made from 35 mm. pyrex tubing. The cup is approximately 140 mm. in length suspended by a glass tubing. Container C is 13 mm. in diameter and 170 mm. long. We have used soft glass test tubes 200 mm.

long. The bottom of C is drawn to a thin, flexible point so that it may be broken to release the cell suspension. The rod supporting C should be of sufficient length to provide a firm grip during lowering. The container may be used repeatedly by simply drawing out a new tip. D is a test tube of 13 mm. diameter and 200 mm. length. The construction and use of the sampling device is apparent; the sample is sucked into D. The gas burettes may be made or purchased. We have found 250 ml. burettes suitable for our work. A saturated salt solution is used in the burettes. The manometer tube F is made of small bore tubing and is approximately 60 cm. in length. Brodie's solution is used in it. The shaking apparatus shown in figure 2 is the International 8892. A wooden framework is bolted on, to which the large Erlenmeyer flasks may be attached. The velocity of the shaking is adjusted to keep the liquid in gentle motion.

STERILIZATION AND ASSEMBLING

The equipment of the reaction flask and the medium are sterilized with steam and allowed to reach the temperature of the incubator. The equipment in the rubber stopper is wrapped in toweling and sterilized. Previous to sterilization the glass tubing is plugged with cotton as indicated in figure 1, the rolls of filter paper are placed in the cups, and clamps and rubber tubing except those of the sampler are removed. A small quantity of glycerol placed on the shaft of A prevents sticking when the tip is to be broken. Pipettes for the addition of constituents of medium, alkali, etc., and extra test tubes for D are also sterilized. Following assembling of L the stopper is sealed with a melted mixture of equal parts of paraffin and beeswax and the apparatus is set up as shown in Plate I on the shaker. The shaker is started and about 15 minutes are allowed for incubation temperature to be attained before the cell suspension is added. Clamps are closed and burette readings, temperature and barometric pressure are recorded. All rubber connections are sealed with the paraffin-beeswax mixture. If the fermentation is to be conducted under anaerobic conditions CO_2 -free nitrogen may be bubbled through the medium, using the sampling device. Burette E should be filled to the zero mark and the liquid lowered after all air has been swept out of L.

REMOVAL OF SAMPLE

First, take the reading as described above, then close J, open H and K, pinch off E, and remove sample by suction. Allow the liquid in the delivery tube to drain back into the reaction flask and close K and H. Open J and E and adjust E until F is balanced and record the reading of the gas burette. If the oxygen consumption is so great as to require resetting of the burette, CO_2 -free oxygen may be introduced through H. By this method the oxygen tension is maintained approximately constant throughout the experiment. If the experiment is under anaerobic conditions, nitrogen should be introduced into H during the removal of samples and resetting of burettes.

DETERMINATION OF CO_2

Two methods have been used for the determination of CO_2 . In one the CO_2 is determined by titration of the alkali in cup A; in the second

method, which requires two reaction vessels for aerobic experiments (one of which contains alkali) the CO_2 is determined by calculation from the observed volumes of gas. The first method probably is more accurate and involves less calculation, but has the disadvantage that only one determination can be made and samples cannot be removed during the course of the experiment. In the second method, since volumes of gas can be read at any time, the CO_2 may be calculated at any desired interval. The procedure for the titrametric method is as follows: exactly 50 ml. of standard alkali are placed in cup A. Approximately 3 N sodium hydroxide has been used by the authors; the strength to be used depends on the quantity of CO_2 to be absorbed. At the conclusion of the experiment the stopper is removed and the outside of the alkali cup and other apparatus in the stopper is rinsed off with distilled water. An evaporating dish about 20 cm. in diameter is used to receive the alkali. The filter paper is placed in the evaporating dish and the alkali poured and washed from the cup. By use of tweezers the filter paper is torn into small pieces. The alkali is filtered by suction through a filter paper on a Buchner funnel. The evaporating dish is thoroughly rinsed with distilled water and the wash water is used to wash the filter paper, which is then pressed water-free after each washing. Washing must be thorough and the final volume of the diluted alkali should be slightly less than 500 ml. The alkali is then diluted to exactly 500 ml. and an aliquot portion is titrated using phenolphthalein as an indicator after adding an excess of saturated barium chloride solution to precipitate the CO_2 as barium carbonate. It is convenient to titrate 50 ml. of the diluted alkali, using a 0.5 N standard solution of HCl. The original alkali is standardized by titration, using the same method as described above, after adding 50 ml. of the alkali to the rolls of filter paper. This is necessary inasmuch as the alkali takes up CO_2 during the manipulation and some may remain in the filter paper. The ml. of N alkali neutralized by the CO_2 divided by 2 gives the mM of CO_2 produced. The oxygen consumed is calculated from the observed decrease in gas volume. The ml. of dry oxygen consumed at N.T.P. divided by 22.4 equals the mM consumed.

The determination of CO_2 by direct measurement of the gas volume is more complicated in that the solubility of CO_2 in water is so large that the dissolved CO_2 must be calculated. This may be done as follows. Let F_1 be the observed change in the gas volume of the flask containing alkali and F_2 that of the flask containing no alkali. The two flasks have the same quantity of medium in each and the reaction is assumed to proceed at equal rates. F_1 is equal to the volume of wet oxygen consumed at the barometric pressure and temperature of the experiment. $F_2 - F_1$ equals the volume of undissolved CO_2 under the same conditions. $F_2 - F_1$ divided by the total volume of gas above the medium equals the fraction of the total gas pressure which is due to the CO_2 . This fraction multiplied by the barometric pressure corrected for vapor pressure divided by 760 multiplied by the solubility (d) of CO_2 times 1000 equals the ml. of CO_2 dissolved per liter of water. The undissolved CO_2 may be calculated on the basis of a liter of medium and this plus the dissolved CO_2 equals the CO_2 produced. If samples are removed at intervals during the experiment the CO_2 must be calculated for each period and the change in the volume of gas above the liquid as well as the volume change of liquid must be taken into account. In experiments, in which the volume of liquid is being

changed frequently, it is convenient to express the calculation on a liter basis.

DISCUSSION

The procedure described in the foregoing paragraphs is based on the assumption that CO_2 is the only gas produced and the method must be modified if this is not the case. Under anaerobic conditions it is possible to determine a second gas for example H_2 by direct measurements when the CO_2 is absorbed in alkali. Aerobically the procedure requires modification. A sample of the gas from the flask L is removed and analyzed and the oxygen consumption calculated from the gas reading when the CO_2 is absorbed in alkali.

As the procedure is given it is assumed that in the presence of alkali a negligible quantity of CO_2 remains bound by the medium. Using phosphate buffer at pH 6.2 or lower this is true; above 6.2 some CO_2 is absorbed and the error increases with increase in alkalinity. If the titrametric method is used the total CO_2 may be computed, the bound CO_2 of the medium being determined by an individual analysis. If NaHCO_3 or any other compound is used in the medium which liberates CO_2 the determination is more involved and the estimation of CO_2 by measurement of gas volume is not feasible, particularly with NaHCO_3 as a buffer. Titrametrically the CO_2 determination may be made if the original quantity of CO_2 present in the medium in the form of carbonate is known. The residual CO_2 of the medium must be determined and this plus that absorbed by the alkali minus the CO_2 of the original medium equals the CO_2 produced by the reaction.

The accuracy of the method and suitability of the apparatus have been determined mainly through its use in the study of bacterial dissimilation of carbohydrates by resting or proliferating cells. The method has proved reliable in aerobic experiments in which a complete determination of products was made. The carbon of the substrate fermented was recovered quantitatively in the determined products. The balance of the oxidized products against the reduced products also was found satisfactory. Although this method of judging the accuracy of the method is indirect it is nevertheless reliable, for it is doubtful whether suitable balances would be obtained if the analyses were in serious error. The titrametric method of determining CO_2 has been used more frequently, but the direct measurement of the gas by volume was found reasonably accurate.

This method of study offers promise of leading to interesting results. The authors have not found reference in the literature to an investigation in which a quantitative determination has been made of the oxygen consumed as well as of the products of dissimilation thus allowing calculation of carbon and oxidation-reduction balances. The usual method of study has been to determine the products and not the oxygen consumed or when the oxygen consumed was determined, the products other than CO_2 were not. Therefore, considerable speculation has been involved in investigations of aerobic dissimilation. The method presented makes available more complete information and eliminates much speculation. The apparatus is suitable for studying quantitatively the effect of different oxygen tensions on the products of dissimilation and thus may be applied to a number of different types of experiments.

SUMMARY

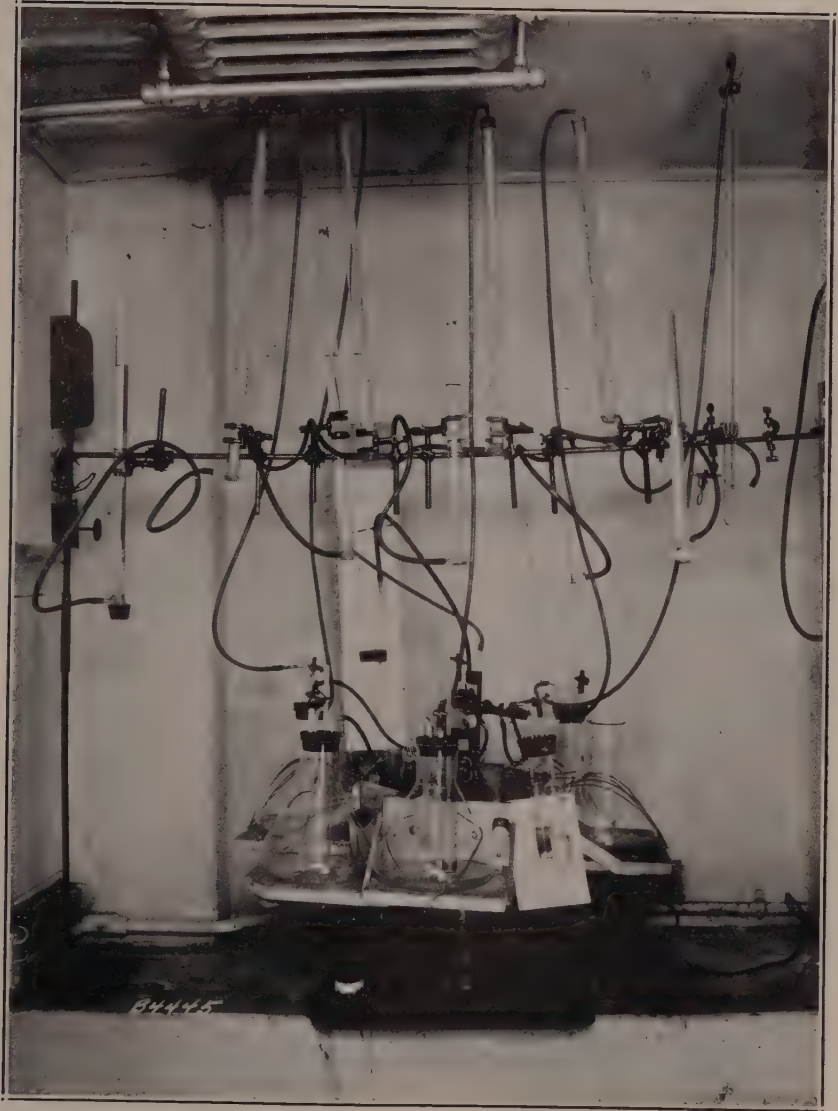
A macro-respirometer is described which permits determination of oxygen consumed, CO_2 produced as well as the non-gaseous products of dissimilation by resting or proliferating cell suspensions. Oxidation-reduction balances may be calculated for aerobic as well as anaerobic dissimilations.

REFERENCE

- DIXON, MALCOLM
1934. Manometric methods. Cambridge University Press.

PLATE I
Macro-respirometer.

PLATE I



METHYLENE BLUE REDUCTION AND OXIDATION-REDUCTION POTENTIAL STUDIES ON MEMBERS OF THE COLON-AEROGENES GROUP OF BACTERIA

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Oxidation-reduction studies of bacterial systems have been given serious consideration in recent years. Until about 1920 such studies were made by means of indicator dyes such as methylene blue, indophenols and derivatives of indigo. Potter (1911) showed the electromotive forces to be different in inoculated media (yeast and *Escherichia coli*) than in media not inoculated.

Gillespie (1920) was the first investigator to interpret the electromotive forces developed in various bacterial cultures in terms of oxidation and reduction.

Cannan, Cohen and Clark (1926) showed that different organisms grown under similar experimental conditions established characteristically different potentials.

Hewitt (1931) presented a very valuable review of investigations concerning potentials developed in bacterial cultures and cited numerous experimental evidences indicating the value of oxidation-reduction studies.

Lindsey and Meckler (1932) described a methylene blue reduction test for distinguishing between *Esch. coli* and *Aerobacter aerogenes* and found potentiometric measurements for two strains to parallel the results obtained by dye reduction. Epstein (1932) found the dye test to be unreliable, but found an apparent relationship between the ability of certain members of the genus *Aerobacter* to reduce methylene blue and the appearance of colonies they formed on eosine methylene blue agar.

The present investigation includes a further study of the methylene blue reduction test and potentiometric studies to determine the following: (1) Relation between oxidation-reduction potentials developed by lactose broth cultures of colon-aerogenes bacteria and their ability to reduce methylene blue. (2) Oxidation-reduction potentials developed in lactose broth cultures of *Escherichia-Aerobacter* bacteria. (3) Relation of oxidation-reduction potentials to growth rates.

METHYLENE BLUE REDUCTION TEST

The results of the methylene blue reduction test carried out on 359 strains of colon-aerogenes bacteria as determined by the addition of 0.5 cc. of 0.1 per cent aqueous methylene blue solution to 10 cc. of 24-hour lactose broth cultures are given in table 1. After adding the dye the tubes were

¹ The writer wishes to express his gratitude and appreciation to Dr. Max Levine, who directed his graduate study, and to Dr. C. H. Werkman for reading the manuscript and offering many valuable suggestions.

vigorously shaken and allowed to stand at room temperature (approximately $25^{\circ}\text{C.} \pm 1.0^{\circ}\text{C.}$) for one hour and then observed for evidence of dye reduction. In recording the results of this test the following key was employed: +++ = complete reduction of the dye with exception of a blue film at the surface of the broth culture; ++ = considerable reduction; + = appreciable reduction; and Sl or — = no appreciable reduction. Heated, cooled and shaken cultures and sterile lactose broth were treated with 0.5 cc. of the dye solution and served as controls.

The results obtained in this study clearly indicated that the methylene blue reduction test does not afford a reliable means of differentiation among the three genera of the colon group. It is interesting to note that

TABLE 1. *Showing results of the methylene blue reduction test*

| Genus | No. of cultures tested | Reduction after one hour | | | | | | | |
|--------------------|------------------------|--------------------------|-------|-----|-------|-----|-------|---------|-------|
| | | +++ | | ++ | | + | | Sl or — | |
| | | No. | Pctg. | No. | Pctg. | No. | Pctg. | No. | Pctg. |
| <i>Escherichia</i> | 0 | 0 | 0.0 | 14 | 29.2 | 17 | 35.4 | 17 | 35.4 |
| <i>Aerobacter</i> | 251 | 166 | 66.1 | 34 | 13.5 | 26 | 10.3 | 25 | 10.0 |
| <i>Citrobacter</i> | 60 | 33 | 55.0 | 14 | 23.4 | 8 | 13.3 | 5 | 8.3 |

+++ = Complete reduction except for blue film at surface.

++ = Considerable reduction. (Half color.)

+ = Appreciable reduction. (Three-quarter color.)

Sl. or — = No appreciable reduction.

an almost equal proportion of the tested *Aerobacter* and *Citrobacter* strains showed ability to reduce methylene blue (giving a +++ reaction). Of the 251 *Aerobacter* cultures tested, 166 or 66.1 per cent reduced the dye within one hour as compared with 33 or 55.0 per cent of the 60 *Citrobacter* cultures giving the same reaction. It is also interesting to note that none of the 48 *Escherichia* strains tested reduced the dye and that 10.0 per cent of the *Aerobacter* strains and 8.3 per cent of the *Citrobacter* strains produced slight or negligible reduction of the dye (giving a Sl or — reaction) as compared to 35.4 per cent of the *Escherichia* strains which gave the same reaction.

CORRELATION OF METHYLENE BLUE REDUCTION WITH COLONY TYPES ON EOSINE METHYLENE BLUE AGAR (LEVINE)

Each of the 359 cultures was studied with respect to characteristics of twenty-four hour colony formed on Difco Levine's dehydrated eosine methylene blue agar. Descriptions of well isolated colonies formed by *Aerobacter* strains are given in table 2 and those by *Escherichia* and *Citrobacter* strains in table 3.

Table 4 presents an analysis of the 359 cultures studied with respect to the relationship of their ability to reduce methylene blue and the types of colonies they formed an eosine methylene blue agar. It is interesting to note that *Citrobacter* cultures on the differential medium resembled

TABLE 2. *Types of Aerobacter colonies on eosine methylene blue agar (Levine formula)*

| Type | After 24 hours at 37° C. | | | | | | |
|---------------------------------|---|---|--|---|--|-----------------------|---|
| | A-I | A-II | A-III | A-IV | A-V | A-VI | A-VII |
| Diameter | 3-5 mm. | 2-4 mm. | 1½-3 mm. | 2-4 mm. | 2-3 mm. | 2-3 mm. | 1½-3 mm. |
| Confluence | Neighboring colonies fuse | Like I | Colonies remain isolated little tendency to fuse | Like I | Like I | Like I | Little tendency to fuse |
| Elevation | Moderately raised | Considerably raised | Slightly raised | Like I | Like III | Like II | Flat or slightly raised |
| Surface | Convex | Like I | Center drops precipitately like crater | Like I | Like I | Like III | Flat or slightly convex |
| Edge | Regular entire | Like I | Like I | Like I | Like I | Like I | Like I |
| Appearance by transmitted light | Light to dark brown centers which are relatively small (less than one-half diameter of the colony) surrounded by a broad grey or pink zone. | Very dark large wine colored centers often three-fourths diameter of colony | Small centers. Less than one-third diameter of colony, light to dark brown or blue | Like I Wine colored or blue centers. Less than one-half diameter of colony | Small centers. Less than one-third diameter of colony. Light to dark brown or blue | Like I Like I | Dark wine or black centers ½-¾ diameter of colony |
| Appearance by reflected light | Metallic sheen not present. Colonies appear glossy and mucoid | Like I, but usually has metallic sheen | Colonies distinct and dull. Crateriform centers usually has metallic luster | Like I but may have weak sheen | Like I but wine colored | Like III but no sheen | Very strong metallic sheen |
| Number of cultures | 63 A-I | 15 A-II | 20 A-III | 88 A-IV | 43 A-V | 8 A-VI | 14 A-VII |

TABLE 3. *Types of Escherichia colonies on eosine methylene blue agar (Levine formula)*

| Type | After 24 hours at 37° C. | | | | |
|---------------------------------|--|--|---|--|--|
| | C-I | C-II | C-III | C-IV | C-V |
| Diameter | 2-3 mm. | 2-4 mm. | 3-4 mm. | 3-4 mm. | 1-2 mm. |
| Confluence | Neighboring colonies do not fuse | Like I | Like I | Like I | Like I |
| Elevation | Slightly raised | Like I | Flat | Like I | Like I |
| Surface | Flat or slightly concave | Slightly convex | Flat, slightly rough | Like II | Convex, smooth, glistening |
| Edge | Regular, entire | Like I | Irregular | Like I | Like I |
| Appearance by transmitted light | Dark, almost black centers which extend three-fourths across diameter of colony, surrounded by a narrow lighter zone | Dark brown or purple centers | Wine centers. Less than one-half diameter of colony | Dark wine colored centers which extend more than three-fourths across diameter of colony | Very light central area. Almost same appearance throughout |
| Appearance by reflected light | Dark, buttonlike, often concentrically ringed, with a greenish sheen | Wine colored when sheen is present it is in area that is halo by transmitted light | Strong metallic sheen | Somewhat mucoid in appearance. Sheen around colony only | Colonies are pink, metallic sheen not present |
| Escherichia cultures | 27 C-I | 11 C-II 11 C-II | 7 C-III | 3 C-IV | 0 C-V |
| Citrobacter cultures | 18 C-I | 39 C-II | 0 C-III | 0 C-IV | 3 C-V |

TABLE 4. Analysis of reduction of methylene blue by colon bacteria and types of colonies formed on eosine methylene blue agar

| Type of colony | No. of cultures | Reaction after one hour | | | | | | | |
|----------------|-----------------|-------------------------|-------|-----|-------|-----|-------|---------|-------|
| | | +++ | | ++ | | + | | Sl or — | |
| | | No. | Pctg. | No. | Pctg. | No. | Pctg. | No. | Pctg. |
| A-I | 63 | 63 | 100.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| A-II | 15 | 2 | 13.3 | 5 | 33.3 | 6 | 40.0 | 2 | 13.3 |
| A-III | 20 | 4 | 20.0 | 8 | 40.0 | 4 | 20.0 | 4 | 20.0 |
| A-IV | 88 | 80 | 91.0 | 6 | 6.8 | 1 | 1.1 | 1 | 1.1 |
| A-V | 43 | 10 | 23.3 | 10 | 23.3 | 10 | 23.3 | 13 | 30.2 |
| A-VI | 8 | 7 | 87.5 | 1 | 12.5 | 0 | 0.0 | 0 | 0.0 |
| A-VII | 14 | 0 | 0.0 | 4 | 28.6 | 5 | 35.7 | 5 | 35.7 |
| *C-I | 18 | 2 | 11.1 | 5 | 37.8 | 6 | 33.3 | 5 | 27.8 |
| *C-II | 39 | 28 | 71.8 | 9 | 23.1 | 2 | 5.1 | 0 | 0.0 |
| *C-V | 3 | 3 | 100.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **C-I | 27 | 0 | 0.0 | 7 | 25.9 | 12 | 44.4 | 8 | 29.6 |
| **C-II | 11 | 0 | 0.0 | 6 | 54.5 | 2 | 18.2 | 3 | 27.3 |
| **C-III | 7 | 0 | 0.0 | 1 | 14.3 | 3 | 42.9 | 3 | 42.9 |
| **C-IV | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| | 359 | | | | | | | | |

+++ Complete reduction except for blue film at surface.

++ Considerable reduction. (Half color.)

+ Appreciable reduction. (Three-quarter color.)

Sl or — No appreciable reduction. (Almost full color.)

* *Citrobacter* cultures.** *Escherichia* cultures.

more closely the typical *Esch. coli* colony than they did the typical *Aerobacter aerogenes* colony. Of the 60 *Citrobacter* strains studied 18, 39 and 3 formed Type C-I, Type C-II and Type C-V colonies, respectively.

Of the 39 *Citrobacter* cultures which formed Type C-II colony 28 or 71.8 per cent gave positive methylene blue tests (+++ reaction), whereas, none of the 11 *Escherichia* cultures forming a similar type colony produced such a reaction.

The data in table 4 further demonstrate that the methylene blue test as performed under the conditions described can not be regarded as a means of differentiating the genera of the colony group of bacteria. There is, however, a striking correlation between certain types of colonies and the dye test. Of the 159 cultures which formed Types A-I, A-IV and A-VI colonies, 150 or more than 94.0 per cent of these strains gave positive methylene blue reduction tests (+++ reactions), whereas, of the 55 cultures which formed Types C-I, C-III and C-IV colonies 53 or more than 96.0 per cent gave completely negative methylene blue reduction tests.

OXIDATION-REDUCTION POTENTIALS DEVELOPED BY TWENTY-FOUR-HOUR LACTOSE BROTH CULTURES

Potentiometric studies of 24-hour lactose broth cultures were made to determine the following: (1) Are the oxidation-reduction potentials characteristics of the genera in the colon-aerogenes group? (2) Are potentiometric readings constant? (3) Is methylene blue in the concentration employed in the methylene blue test a reliable indicator of oxidation-reduction potentials?

The potentiometric measurements for each of 45 cultures were made in duplicate. Figure 1 demonstrates a striking difference in the oxidation-reduction potentials developed by *Aerobacter* cultures forming typical *Aerobacter aerogenes* (Type A-I) colonies on eosine methylene blue agar and those developed by *Escherichia* cultures. With the exception of culture No. 24, the Eh of the *Aerobacter* cultures did not vary more than 0.01 volt during the period of observation.

Figure 2 shows a variation of Eh values produced by different *Aerobacter* cultures. It will be noted that the oxidation-reduction potentials produced by three *Aerobacter* lactose broth cultures (Numbers 32, 192a and 257sA) were more positive than the potential established by *Escherichia* culture No. C₃₃. In general, the potential readings were reasonably constant.

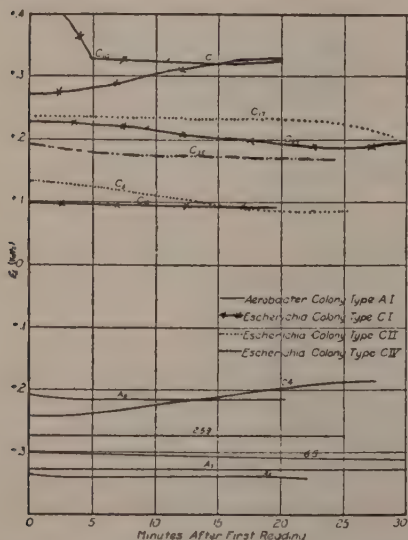


Fig. 1. Oxidation-reduction potentials of 24-hour lactose broth cultures.

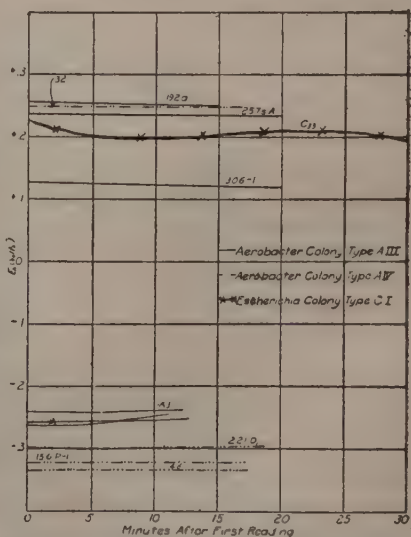


Fig. 2. Oxidation-reduction potentials of 24-hour lactose broth cultures.

RELATION OF POTENTIOMETRIC MEASUREMENTS TO THE METHYLENE BLUE TEST

In order to ascertain the reliability of methylene blue in the concentration employed as an indicator of oxidation-reduction potentials, 10 cc. sterile standard lactose broth in test tubes (18 x 150 mm.) were inoculated in triplicate with a loopful (4 mm. in diameter) of an 18-hour

nutrient broth culture and incubated for 24 hours at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. At the expiration of the incubation period, the potentiometric measurement for each culture was made in duplicate and at the same time the methylene blue test was run on the third culture. Reasonable agreement was found in the oxidation reduction potentials for each set of duplicate run.

Table 5 was drawn up to show the relation of the potentiometric measurements to the methylene blue test and to indicate the reliability of methylene blue in the concentration employed in the dye test as an indicator of oxidation-reduction potentials of lactose broth cultures. The Eh values listed were those observed 10 minutes after the first reading was recorded. The mid-point values (E'_{0}) of the methylene blue-methylene white systems at the given pH conditions were determined by graphing the results of Clark, Cohen and Gibbs (1925, tables 17 and 18) and interpolating.

According to Hewitt (1931) at constant pH, the potential of a 50 per cent oxidized methylene blue solution (E'_{0}) (half color) is about 0.060 volt more positive than the potential of the dye solution when one per cent oxidized (almost colorless). When the methylene blue is 98 per cent oxidized (almost full color) the potential is about 0.060 volt more positive than the potential at the methylene blue-methylene white midpoint (E'_{0}). Allowing ± 0.040 volt variation which might take place from the time the dye is added to the time the results of the methylene blue reduction test (one hour) is read, a $+++$ reaction of the dye test should coincide with a potential value of 0.10 volt more negative than the oxidation reduction potential of the methylene blue-methylene white midpoint E'_{0} and $S1$ or $-$ reaction should be 0.10 volt more positive than the E'_{0} . Such a relationship is considered a parallelism of potentiometric measurement with the methylene blue reduction test.

Table 5 shows that an agreement of the results of the potentiometric measurements and the methylene blue test was obtained with 33 of the 45 cultures tested. In seven instances (Cultures 71 small, 213 aty, 9₁, 183, 134, 110 red₁ and 226) the Eh was more than 0.20 volt more negative than the E'_{0} and positive results ($+++$ reaction) were not obtained with the dye test showing that the bacterial cultures in question produced potentials of such small capacities that the concentration of the dye used was able to take on all of the escaping electrons in the system without being appreciably reduced. In five instances (Aerobacter cultures 192a and 257sA and Escherichia cultures C₁, C₁₀ and C₁₇) the methylene blue test reactions were either $+$ or $++$ corresponding to about 75 and 50 per cent oxidation, respectively, and the Eh values were more positive than the E'_{0} values by more than 0.120 volt, indicating the reduction of the dye was probably caused by factors other than the existing oxidation-reduction potential of the bacterial cultures at the time of the introduction of the dye.

OXIDATION-REDUCTION POTENTIALS DEVELOPED IN LACTOSE BROTH CULTURES OF ESCHERICHIA-AEROBACTER BACTERIA

The potential-time changes developed by cultures of Escherichia-Aerobacter bacteria in lactose broth were followed by electrode measurements in the following manner: Thirty cc. sterile lactose broth in 200 x 25 mm. cotton-plugged test-tubes were inoculated with 0.1 cc. of 24-hour

TABLE 5. *Relation of oxidation-reduction potentials to methylene blue reduction test. Twenty-four-hour lactose broth cultures*

| Culture | Colony type on E.M.B. agar | 24-hr. lactose broth | | M.B.-M.W. midpoint E' | Deviation in volts of Eh from E' | M.B. test | Agreement of M.B. test and Eh |
|----------------------|----------------------------|----------------------|-------|-----------------------|----------------------------------|-----------|-------------------------------|
| | | pH | Eh | | | | |
| A ₆ | AI | 5.35 | -.216 | +.080 | -.296 | +++ | + |
| A ₇ | | 5.30 | -.327 | +.082 | -.409 | +++ | + |
| A ₉ | | 5.50 | -.340 | +.073 | -.413 | +++ | + |
| 24 | | 5.75 | -.229 | +.059 | -.288 | +++ | + |
| 65 | | 5.45 | -.304 | +.075 | -.379 | +++ | + |
| 259 | | 5.25 | -.274 | +.084 | -.358 | +++ | + |
| 66A | (s) AII | 5.25 | -.287 | +.084 | -.371 | +++ | + |
| 71 small | | 4.88 | -.264 | +.107 | -.371 | + | - |
| 121 small | | +4.80 | +.149 | +.115 | +.034 | Sl | + |
| 198-2 | | 4.85 | +.190 | +.112 | +.078 | Sl | + |
| 213 aty | | 5.00 | -.106 | +.101 | -.207 | ++ | - |
| 9 ₁ | (s) | 5.04 | -.244 | +.100 | -.344 | + | - |
| 183 | (s) AIII | 4.90 | -.238 | +.107 | -.345 | + | - |
| 192a | | 4.73 | +.254 | +.121 | +.133 | + | - |
| 257sA | | 5.12 | +.237 | +.093 | +.144 | + | - |
| 306-1 | | 5.35 | +.117 | +.090 | +.037 | + | + |
| A ₁ | | 5.25 | +.029 | +.084 | -.055 | +++ | + |
| A ₁₀ | (s) AIV | 5.45 | -.279 | +.075 | -.354 | +++ | + |
| 6 | | 5.70 | -.320 | +.062 | -.382 | +++ | + |
| 32 | | 4.90 | +.249 | +.107 | +.142 | Sl | + |
| 156P-1 | | 5.75 | -.322 | +.059 | -.381 | +++ | + |
| 188r ₂ B | | 5.60 | -.325 | +.066 | -.391 | +++ | + |
| 221D ₁ | (s) | 5.35 | -.299 | +.080 | -.398 | +++ | + |
| 46 | (s) AV | 5.70 | -.334 | +.062 | -.396 | +++ | + |
| 253 | | 5.40 | -.260 | +.077 | -.337 | +++ | + |
| 5R ₁ | | 5.05 | +.368 | +.100 | +.268 | Sl | + |
| 19A | (s) AV | 5.45 | -.346 | +.075 | -.421 | +++ | + |
| 134 | | 5.47 | -.298 | +.074 | -.372 | + | - |
| 110A ₂ | | 5.60 | -.315 | +.066 | -.381 | +++ | + |
| 156WP | AVI | 5.50 | -.341 | +.073 | -.414 | +++ | + |
| 110 red ₁ | (s) | 5.10 | -.239 | +.093 | -.332 | Sl | - |
| 116 | (s) | 5.02 | +.188 | +.101 | +.086 | + | + |
| 197D ₁ | AVII | 5.10 | +.102 | +.093 | +.009 | Sl | + |
| 226 | (s) | 5.20 | -.237 | +.088 | -.325 | + | - |
| *C ₁ | CI | 4.80 | +.305 | +.115 | +.190 | + | - |
| C ₅ | CH | 4.80 | +.115 | +.115 | +.000 | ++ | + |
| C10 | CI | 5.00 | +.324 | +.101 | +.223 | ++ | - |
| C13 | CI | 4.80 | +.096 | +.115 | -.019 | ++ | + |
| C17 | CH | 4.90 | +.234 | +.107 | +.127 | ++ | - |
| C33 | CI | 4.90 | +.215 | +.107 | +.108 | + | + |
| C35 | CIV | 4.80 | +.175 | +.115 | +.060 | + | + |
| **H ₁ | CV | 5.40 | -.386 | +.077 | -.463 | +++ | + |
| H ₂ | CII | 5.20 | -.217 | +.088 | -.305 | +++ | + |
| H ₃ | CV | 5.40 | -.260 | +.077 | -.337 | +++ | + |
| H ₄ | CII | 5.30 | -.153 | +.082 | -.235 | +++ | + |

(s) = Metallic sheen on colony.

* = Escherichia cultures.

** = Citrobacter cultures.

E' = Potential 50 per cent reduction of methylene blue at the given pH.

+++ = Complete reduction.

++ = Considerable reduction.

+ = Appreciable reduction.

Sl or - = Slight or no reduction.

lactose broth cultures and incubated at 37° C. The incubator was specially built for potentiometric studies and was conveniently equipped with a saturated KCl calomel half cell. The door of the incubator had two sliding portal openings and a small glass window which made it possible for a worker to connect the culture system to the reference cell and to a vacuum tube potentiometer kept at room temperature.

Prior to inoculations the lactose broth was held at 37° C. for 48 hours in order to detect the presence of any possible contaminations and to permit stabilization of potential. Two strains were selected for this study. One (A_0) was an *Aerobacter* culture isolated from soil and the other (C_{33}) was an *Escherichia* strain isolated from human dejecta. On E. M.B. agar, each formed colonies typical for *A. aerogenes* (Type A-I) and *Esch. coli* (Type C-I), respectively.

The pH and Eh values of the medium before inoculation were 6.92 and + 0.306, respectively. In making Eh determinations a culture tube was selected at random and connected to the calomel half cell and potentiometer. Precautions were taken to avoid shaking the culture tubes. Ten to 15 minutes were allowed to elapse before a reading was made and recorded. Immediately thereafter the culture was removed from the incubator, allowed to cool to room temperature (25° C. \pm 1.0° C.) and its pH value determined by the glass electrode method.

The results of this experiment are presented in figure 3. The marked difference in Eh and pH curves for each organism is noticeable. Both cultures show decided negative drifts in potential during the first nine hours of growth, accompanied by lowering pH values. Thereafter, the curves for each culture differ. In contrast to the slight positive trend of the *Aerobacter* strain, the *Escherichia* culture shows a sharp rise toward positive values in potential. The striking difference of potential is more clearly shown in table 6, in which the Eh of each culture is compared with the Eh of the methylene blue-methylene white midpoint, E'_0 , at the respective pH values of the cultures in question. The observations made after 9 and 12 hours growth show the Eh of both the *Aerobacter* and *Escherichia* cultures to be about equally more negative than the potential of the E'_0 . Later, however, the potential of the *Escherichia* culture became more positive than the E'_0 . Later, 28 hours the potential was 69 millivolts more positive than the E'_0 and after 120 hours it was 253 millivolts more positive. The potential of the *Aerobacter* culture maintained its negative character showing an Eh 256 millivolts more negative than the E'_0 after 120 hours.

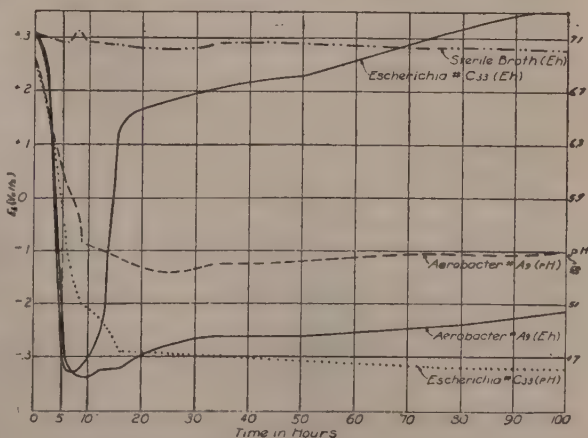


Fig. 3. Ph and oxidation-reduction potential curves of *Escherichia*-*Aerobacter* lactose broth cultures.

TABLE 6. Relation of oxidation-reduction potentials of *Escherichia-Aerobacter* lactose broth cultures to methylene blue-methylene white midpoint, E' .

| Time in hours | Control sterile lactose broth | | Aerobacter No. A9 | | | | Escherichia No. C ₁₃ | | | |
|---------------------|----------------------------------|------|-------------------|-------|---|--------------------------------------|--|---|------|------|
| | | | Eh | Ph | *Methylene blue methylene white midpoint, E' | Deviation Eh from E' (volts) | Deviation of Eh from E' (volts) | **Methy- lene blue methylene white midpoint E' | | |
| | Eh | Ph | | | | | | | | |
| 0 | +306 | 6.92 | | | | +203 | +183 | +029 | 6.45 | +212 |
| 2.5 | +295 | 6.90 | +231 | 6.50 | +028 | -361 | -391 | +066 | 5.59 | -325 |
| 6.0 | +290 | 6.89 | -314 | 6.00 | +047 | -361 | -418 | +089 | 5.19 | -329 |
| 8.0 | +310 | 6.88 | -337 | 5.80 | +056 | -393 | -412 | +094 | 5.10 | -318 |
| 9.0 | +293 | 6.90 | -341 | 5.55 | +064 | -405 | -371 | +101 | 5.0 | -270 |
| 12.0 | +290 | 6.93 | -326 | 5.50 | +071 | -397 | +021 | +121 | 4.75 | +142 |
| 16.0 | +285 | 6.91 | -321 | 5.45 | +075 | -396 | +069 | +121 | 4.72 | +190 |
| 28.0 | +281 | 6.92 | -273 | 5.35 | +079 | -352 | +084 | +121 | 4.76 | +205 |
| 34.0 | +290 | 6.90 | -260 | 5.41 | +077 | -377 | +107 | +123 | 4.68 | +230 |
| 50.0 | +288 | 6.91 | -263 | 5.38 | +077 | -340 | +179 | +126 | 4.63 | +305 |
| 75 | +281 | 6.88 | -241 | 5.48 | +071 | -312 | +235 | +127 | 4.62 | +362 |
| 105 | +274 | 6.90 | -208 | 5.55 | +064 | -272 | +253 | +128 | 4.59 | +381 |
| 120 | +269 | 6.89 | -195 | 5.70 | +061 | -256 | | | | |

* Midpoint at pH indicated for No. A9.

** Midpoint at pH indicated for No. C₁₃.

The curves are also markedly different. As with the Eh potential, both organisms rapidly lowered the pH of the medium during the first 9 hours of growth, the values reached at that time were 5.50 and 5.10 for the *Aerobacter* and *Escherichia* cultures, respectively. The *Escherichia* culture continued a rather sharp decline of pH value for the next 7 hours when a pH value of 4.75 was reached and then showed a gradual decline until a pH value of 4.59 was reached at the end of 120 hours of growth. On the other hand, the *Aerobacter* culture showed a much lesser decline in pH value from the 9-hour period to the 16-hour period and reached a minimum of 5.35 after 28 hours of growth. The trend of the curve then assumed a gradual rise until a pH value of 5.70 was reached after 120 hours. The pH curves are in accord with the explanation of Clark and Lubs (1915) with reference to the activity of *Esch. coli* and *A. aerogenes* cultures in sugar broths. The former increased the hydrogen ion concentration to a point where it becomes suicidal and growth stops, while the latter is capable of converting the acids and consequently grows for a much longer time.

The character of the Eh curves may be best explained as being due to the difference in the metabolic activities of the two organisms with reference to the balance of reducing and oxidizing substances produced. It seems that during the first 9 hours of growth both organisms produce a reducing condition in the substrate. Thereafter, the *Escherichia* culture showed an increase in oxidizing substances, as is evidenced by a rise in potential from -0.270 volt after 12 hours of growth to $+0.190$ and $+0.362$ volts after 28 and 105 hours. On the other hand, the *Aerobacter* culture retains its reducing conditions for a period of up to 120 hours (the length of the experiment), at which time the Eh value was -0.195 .

RELATION OF OXIDATION-REDUCTION POTENTIALS TO GROWTH RATES

An attempt to uncover a more specific explanation of the oxidation-reduction potential in lactose broth was made by determining growth curves in 30 cc. quantities of medium in 200 x 25 mm. cotton-plugged test-tubes inoculated with 0.1 cc. of 24-hour lactose broth cultures. Bacterial counts were made at once and subsequent frequent intervals.

Plates were made in duplicate on standard nutrient agar, incubated at 37° C. for 48 hours. Colony counts were made in a Buck colony counting box. The results are given in table 7.

Figure 4 shows Eh and growth curves for *Aerobacter* culture No.

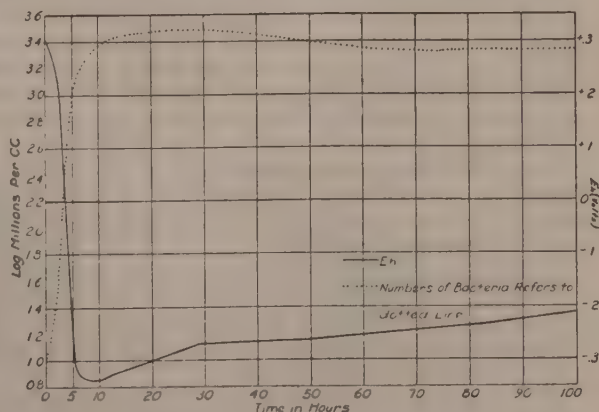


Fig. 4. Relation of Eh to numbers of bacteria in lactose broth *Aerobacter* culture No. A9.

TABLE 7. *Numbers of bacteria in lactose broth*

| Culture | Aerobacter (A_0) | Escherichia (C_{33}) |
|---------------|------------------------------------|--------------------------|
| Time in hours | Average number of bacteria per cc. | |
| 0 | 1,040,750 | 850,000 |
| 2 | 3,100,000 | 2,600,000 |
| 4 | 37,000,000 | 38,500,000 |
| 6 | 140,500,000 | 138,500,000 |
| 8 | 166,000,000 | 154,000,000 |
| 10 | 201,000,000 | 176,000,000 |
| 12 | 259,500,000 | 187,000,000 |
| 22 | 302,500,000 | 204,500,000 |
| 30 | 305,500,000 | 218,250,000 |
| 50 | 245,000,000 | 143,500,000 |
| 75 | 221,500,000 | 125,500,000 |
| 103 | 251,000,000 | 91,000,000 |
| 120 | 216,000,000 | 35,000,000 |

A_0 , and present a striking relationship. The growth phase does not show a pronounced lag phase due to the large inoculum and the failure to determine counts after one hour of growth.

The logarithmic growth phase apparently occurred from the second to the fifth or sixth hour and the phase of negative growth acceleration lapsed into the maximum stationary phase at about the twelfth hour, which seemed to last until the thirtieth hour. Thereafter, the phase of a very gradual accelerated death set in. The slow fall in potential during the first 2.5 hours, followed by the rapid decrease up to the sixth hour,

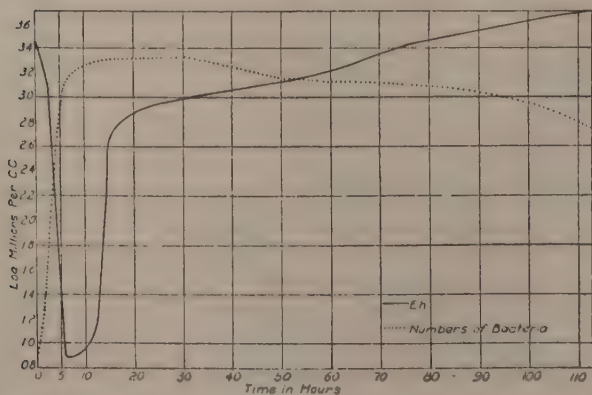


Fig. 5. Relation of Eh to numbers of bacteria in lactose broth Escherichia culture No. C33.

corresponds inversely to the slow rise followed by the sharp incline of the growth curve during the same period of time. The continued fall in potential during the next three hours inversely corresponds to the slow rise in the growth curve (negative growth acceleration phase). The sudden reversal in direction of the potential occurs at about the time (after ninth hour of growth) when the negative

growth acceleration is at its maximum. During this period, the activity of the cells is decreased. The gradual rise in potential which takes place from this point up to the 120th hour seems to be related to the decreased activity of the cells.

Figure 5 shows Eh and growth curves for *Escherichia* culture No. 33. The relationship of the Eh curve to the growth curve during the lag, logarithmic and negative growth acceleration phase is similar to the relationship of these curves for culture A₉. The rise in potential during the beginning of the maximum stationary phase (from the twelfth to the sixteenth hour) is exceedingly sharp. A possible explanation for this phenomenon may be the decrease of activity of the bacteria. The trend, thereafter, slackens. The phase of accelerated death begins somewhere between the thirtieth and fiftieth hour and the decrease in numbers of bacteria per cc. during this phase seems to correspond to the rise in the oxidation-reduction potential.

From the observations made it seems possible that the rate of growth is an important factor in determining the potentials developed by lactose broth cultures exposed to air.

SUMMARY

The ability of 24-hour lactose broth cultures to reduce methylene blue was tested on 359 strains of the colon-aerogenes group of bacteria. The technique involved the addition of 0.5 cc. of 0.1 per cent aqueous methylene blue solution to 10 cc. of lactose broth culture tubes (1.6 x 15 cm.), vigorously shaking the mixture and then allowing the tubes to stand at room temperature (approximately 25° C. \pm 1.0° C.) for one hour and then observing for evidence of dye reduction. The results showed that there was no sharp differentiation of genera. There was, however, a striking correlation between the relative abilities of certain cultures to reduce methylene blue and the character of colony they formed on eosine methylene blue agar (Levine formula).

Potentiometric studies on 45 strains showed that the potential (Eh) developed by 24-hour lactose broth cultures were definitely positive with all of the *Escherichia* strains (7) tested, but were not negative with all of the *Aerobacter* cultures (34). In 12 instances the potential measurements did not parallel the results of the dye reduction, thereby indicating that methylene blue in the concentration used cannot be regarded as a reliable indicator for detecting oxidation-reduction potentials developed in bacterial cultures.

All *Aerobacter* strains forming typical *Aerobacter aerogenes* colonies on eosine methylene blue agar and over 90.0 per cent of the *Aerobacter* strains which formed bluish mucoid colonies on the same medium gave positive methylene blue reduction tests and produced decided negative oxidation-reduction potentials in 24-hour lactose broth cultures. Eosine methylene blue agar, therefore, is a good index and a much easier one than the others for indicating how the strains in question will behave in lactose broth with respect to development of oxidation-reduction potential.

Potential time courses developed in lactose broth cultures were followed by electrode measurements. The strains employed produced either typical *Aerobacter aerogenes* or typical *Escherichia coli* colonies on eosine

methylene blue agar. The Eh curve produced by *Aerobacter* culture was markedly different from the one produced by *Escherichia* culture. Experimental evidence is presented to show that the rate of growth or activity of the cells in question is an important factor in determining the potentials developed by lactose broth cultures exposed to air.

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LABORATORY REPORT ON TESTS TO DETERMINE CAUSES OF STRETCH³ AND SLIPPAGE OF YARNS IN CERTAIN WOOL AND SILK FABRICS¹

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Standardized fabrics are difficult to obtain from the general market. New fabrics increase in number each year, consequently there is lacking a basis for comparison of the important features of a fabric.

Of the fabrics which appear on the market, the textile worker is aware of wide variation in their construction. The value of a fabric to the consumer is based largely upon what is commonly known as the wearing quality of the fabric.

From observations made over a long period of time it is reasonable to believe that some of the factors which influence the amount of stretch and slippage of yarns in a fabric are: number of twists per inch, number of yarns per inch—warp and filling, and breaking strength. In addition, a fabric to be satisfactory must be so constructed as to admit of dry cleaning and laundering.

So with the consciousness of these problems, an attempt was made to study some of the factors involved in the construction of woolen and silk fabrics.

The investigations herein reported are the results of laboratory studies on (A) the factors affecting the stretch and slippage of yarns in certain wool and silk fabrics; (B) elongation and slippage of yarns in certain weighted silks. Only the experimental data of problems I and II are recorded.

Glanton (2) says that the three principal factors which make for shrinkage of wool are (a) curly fibers, (b) lack of combing, even though the fibers are relatively straight, and (c) close weaves, which result in the matting of serrations on the wool fibers. Hence, this shrinkage is increased if the yarns lack twist or have not been well spun.

Johnson (3) says that the slippage of yarns encountered in light weight silks and loosely woven fabrics is caused by the warp sliding over the smooth filling. It occurs in garments chiefly at elbows or at shoulders or other places of stress.

Silk filling generally consists of tram, which possesses only enough twist to hold individual silk filaments together after the yarn has been thrown. Tram, because of its lack of twist, presents a very smooth surface; under the influence of strain the warp slips so that the weave becomes distorted.

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³ Stretch and elongation are synonymous. Slippage of yarns is the pulling apart of yarns under tension.

According to Edgerton (1) the slippage of yarns at points of strain in garments is one of the chief causes of the high cost of the consumer's adjustment claims with their resultant loss to the retailer. Yarns slip at the elbows, shoulder seams, side seams of skirts and other points of stress. The yarn slips because the material is woven too loosely or the thread are not sufficiently stayed in weaving. The smooth yarns are more likely to slip than the rougher yarns. In garments cut the wrong way of the fabric, the tendency for yarns to slip will be increased. If garments are cut too small there will be pull at the points of strain, which will increase the tendency for yarns to slip at the seams.

Meyer and Whitlock (4), at the University of Illinois, studied the problem of slippage of yarns in the silk crêpe fabrics. These authors came to the conclusion that the most outstanding factor in overcoming yarn slippage is in the construction of the filling thread. The corkscrew twist seems to be the only preventive.

EXPERIMENTAL WORK

The greater proportion of the fabrics used in Problem I were contributed by woolen and silk manufacturers. Fourteen representative dyed fabrics were used, eight woolen and six silk fabrics.

All physical tests on the fabrics were made under standard conditions of temperature (70° F.) and humidity (65 per cent relative humidity).

The weight of each fabric was estimated by removing three specimens, each four inches long, from the entire width of the fabric. These were conditioned and weighed to the nearest milligram. The average weight, calculated in ounces per square yard, was recorded as the weight of the fabric (5).

The width of the fabric was determined by spreading it, without tension, on a flat surface and measuring the distance between selvages. Three measurements at different parts of the fabric were taken and the average recorded as the width.

The thickness of the fabric was determined by means of an automatic micrometer which pressed on a circle of fabric three-fourths inch in diameter with a pressure of six ounces. No measurements were taken within six inches of the selvage. Ten determinations were made at different places on the fabric and the average recorded as the thickness of the fabric.

The number of yarns per inch was counted with a Lowinson thread counter. The warp yarns were counted in five places, six inches or more apart, and not less than eight inches from the selvage. The average number of yarns per inch was taken as the warp yarn count. This procedure was repeated for the filling yarns.

Yarn count was determined by conditioning and weighing to the nearest milligram, ten-yard lengths of both warp and filling yarns. The averages were expressed in thousands yards per pound.

Yarn twist was determined by means of the Precision Twist Counter. This machine records the number of twists for a two-inch piece of yarn and indicates the direction of the twist. Ten determinations were taken for both warp and filling yarns and the average recorded as the number of twists per inch.

Folding endurance was determined by use of the M. I. T. Folding Endurance Tester, which exerted a tension of one kilogram on the fabric. Ten specimens, each one centimeter by ten centimeters, were conditioned and classified in the machine and bent 135 degrees on either side of the no-fold position. The average folding endurance of the ten specimens was determined.

The fabrics were laundered by the method given by the American Association of Textile Chemists and Colorists. The laundering was done under home laundry conditions. Distilled water at 100° F. was used all through the laundering process. Five grams of pure olive oil soap was dissolved in one liter of distilled water. The silks were washed in this suds for not more than five minutes by squeezing and kneading. They were rinsed three times, then rolled in a towel until almost dry and ironed with a moderately hot iron.

The woolen fabrics were washed in the same manner, but were allowed to dry in a current of air at room temperature for 20 minutes and then ironed.

The fabrics were laundered twice. After taking off a portion of each for testing, they were again laundered twice. This process was repeated through eight launderings.

The fabrics were dry cleaned with a load of garments, each load being run for 45 minutes. They were transferred to an extractor, which removed the solvent by centrifugal force. They were next put into a tumbler, which removed the odor by forcing hot dry air through them. Steaming and pressing were done on a steam table, live steam being forced through the fabrics during the pressing. The fabrics were cleaned twice and portions were cut off for testing; they were again cleaned twice and the portions tested. This process was repeated through eight dry cleanings.

Before the fabrics were laundered or dry cleaned, yarns were drawn in the material one inch from the edge of a square piece of fabric. Three measurements were taken in the direction of the warp, and three in the direction of the filling yarns, and the averages then calculated. Measurements of these squares were taken after each series of dry cleanings and launderings and the percentage change in length calculated.

The breaking strength of the fabric was determined by means of the Scott Universal Tester. This machine records the number of pounds required to break a specimen of the fabric. The strip method was used in testing. Five specimens one inch by seven inches were cut in both warp and filling directions of the fabric. No two specimens had the same set of yarns and no specimens were cut nearer than one inch to the selvage. The results were averaged and the breaking strength recorded in pounds.

Plain, French, and flat-fell seams, run in both warp and filling direction, were tested for breaking strength, slippage of yarns and for elongation after the last laundering and dry cleaning.

DISCUSSION OF DATA

The results of the two problems have been combined because of the similarity of method and data obtained. The woolens and silks tested varied greatly in breaking strength after the cleaning processes. This was especially true of the silks. However, the breaking strength of the fabrics, when considered as a group, decreased comparatively little from cleaning, as shown in figures 3 and 4 of Problem I and figure 2 of Problem II.

TABLE 1. *Fabric analysis* (Problem I. Factors affecting stretch and slippage of yarns in certain wool and silk fabrics)

| | | | Number yarns | | Twist | | Breaking strength | |
|----------------------------|---------------|---------|-----------------|--------------|-------|--------------|----------------------|--------------|
| Fabric | Width inch | Weave | inch | | inch | | pounds | |
| | | | warp | Fill- ing | warp | Fill- ing | warp | Fill- ing |
| 1. Twilled suiting | 54.00 | twill | 49 | 44 | 6L | 9L | 19 | 21 |
| 2. Angora suiting | 53.75 | novelty | 45 | 36 | 12L | 15L | 15 | 9 |
| 3. Wool crêpe | 51.50 | cord | 27 | 36 | / 22L | / 23L | 24 | 22 |
| | | plain | | | \ 22R | \ 22R | | |
| 4. Flannel | 53.50 | plain | 37 | 37 | 20R | 15L | 18 | 15 |
| 5. Wool crêpe | 54.00 | plain | 41 | 36 | 11R | 9R | 20 | 16 |
| 6. Wool crêpe | 53.50 | plain | 45 | 35 | 16R | 12R | 18 | 17 |
| 7. Wool crêpe | 54.00 | plain | 47 | 40 | 13R | 14R | 34 | 17 |
| 8. Suiting | 54.00 | plain | 43 | 37 | / 17R | / 19R | 18 | 13 |
| | | | | | \ 17L | \ 19L | | |
| <u>Silk</u> | | | | | | | | |
| 9. Twill (pure dye) | 27.00 | twill | 134 | 98 | 8R | 8L | 52 | 87 |
| 10. Flat crêpe | 39.00 | plain | 88 | 89 | 0 | 21R | 54 | 31 |
| 11. Flat crêpe | 38.75 | plain | 66 | 86 | 0 | 23L | 40 | 27 |
| 12. W. cord weave crêpe | 39.00 | cord | 146 | 56 | 0 | 46R | 69 | 24 |
| 13. W. twill | 38.75 | twill | 77 | 81 | 0 | 19R | 41 | 18 |
| 14. Rajah | 27.00 | plain | 66 | 71 | 2R | 0 | 49 | 72 |

R—Right hand twist.

L—Left hand twist.

W—Weighted silk.

RELATION BETWEEN NUMBER OF DRY CLEANINGS OR LAUNDERINGS AND ELONGATION

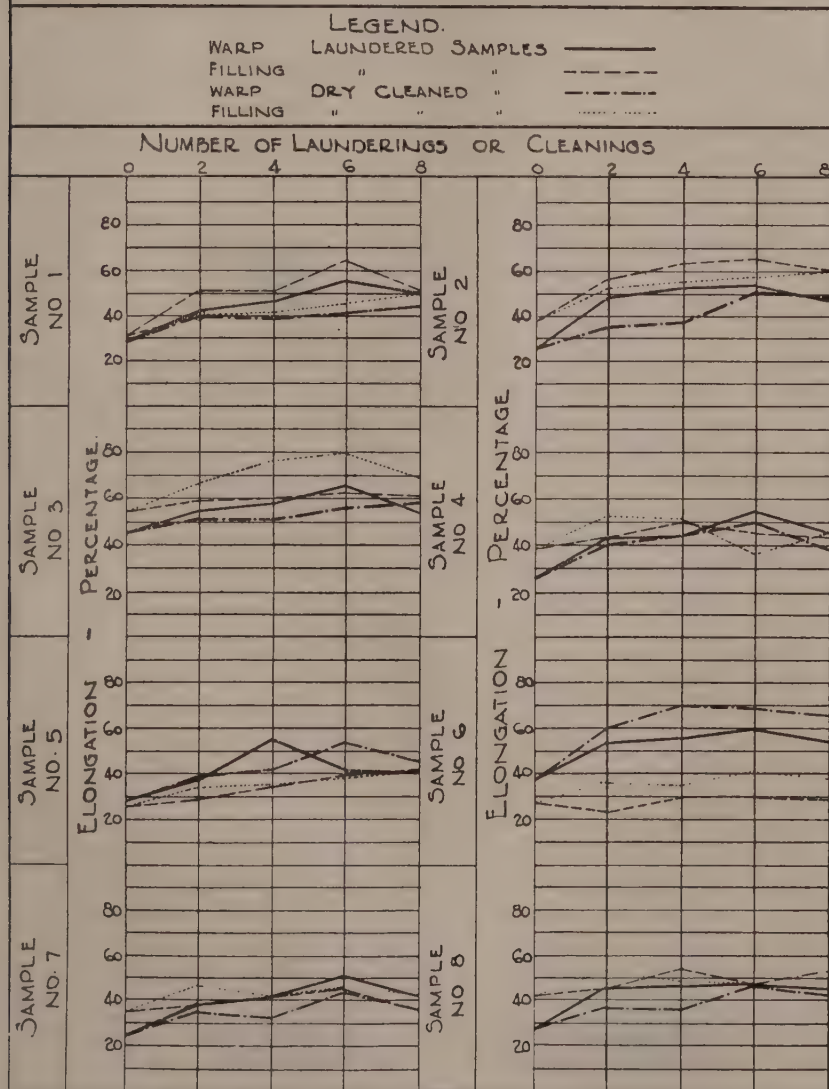


FIG. I
(Problem I)

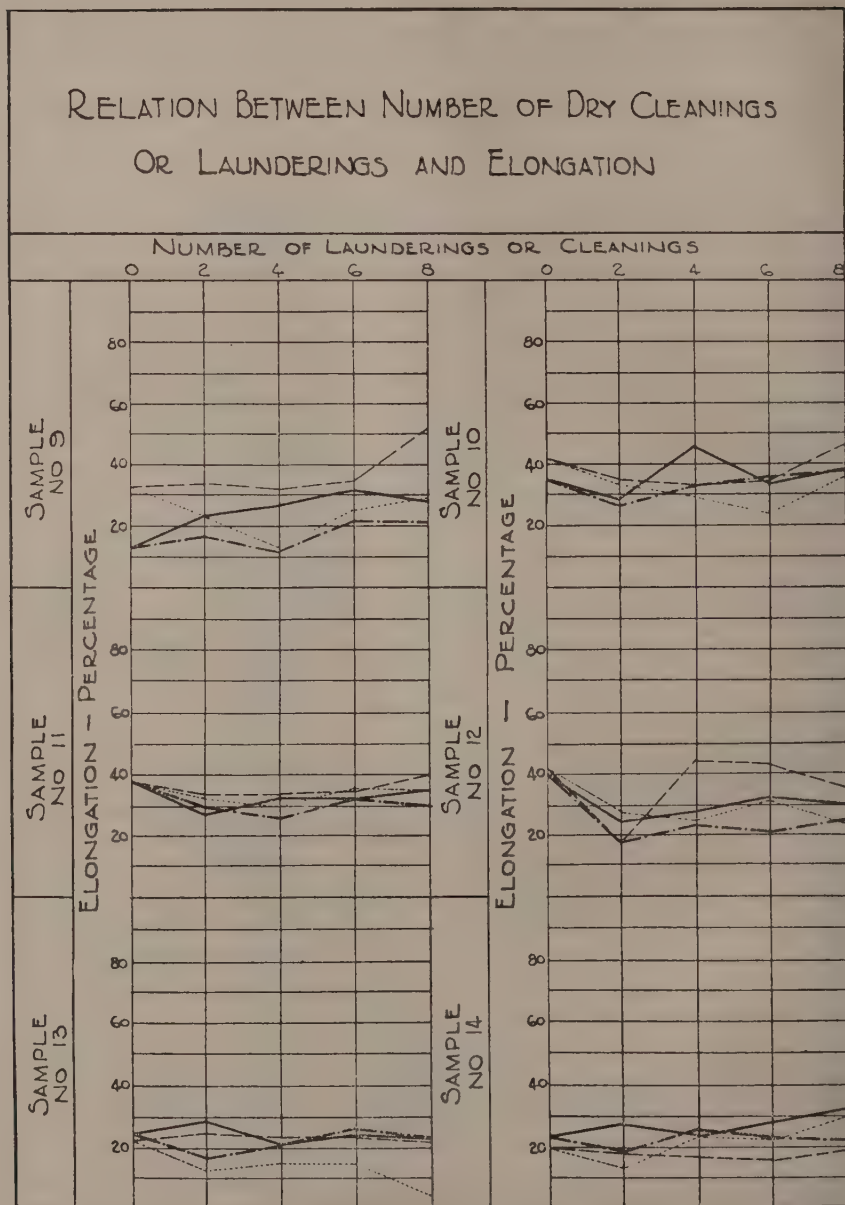


FIG. 2
(Problem I)

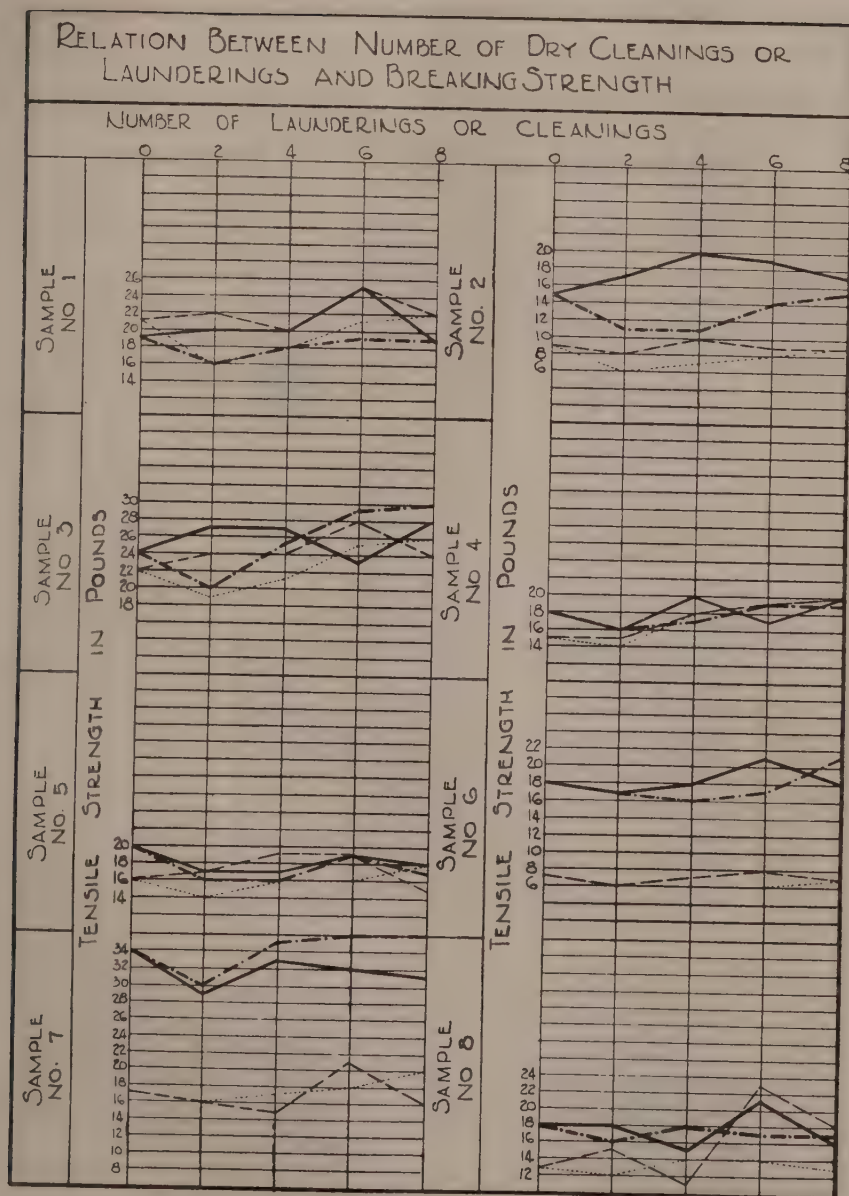


FIG. 3
(Problem I)

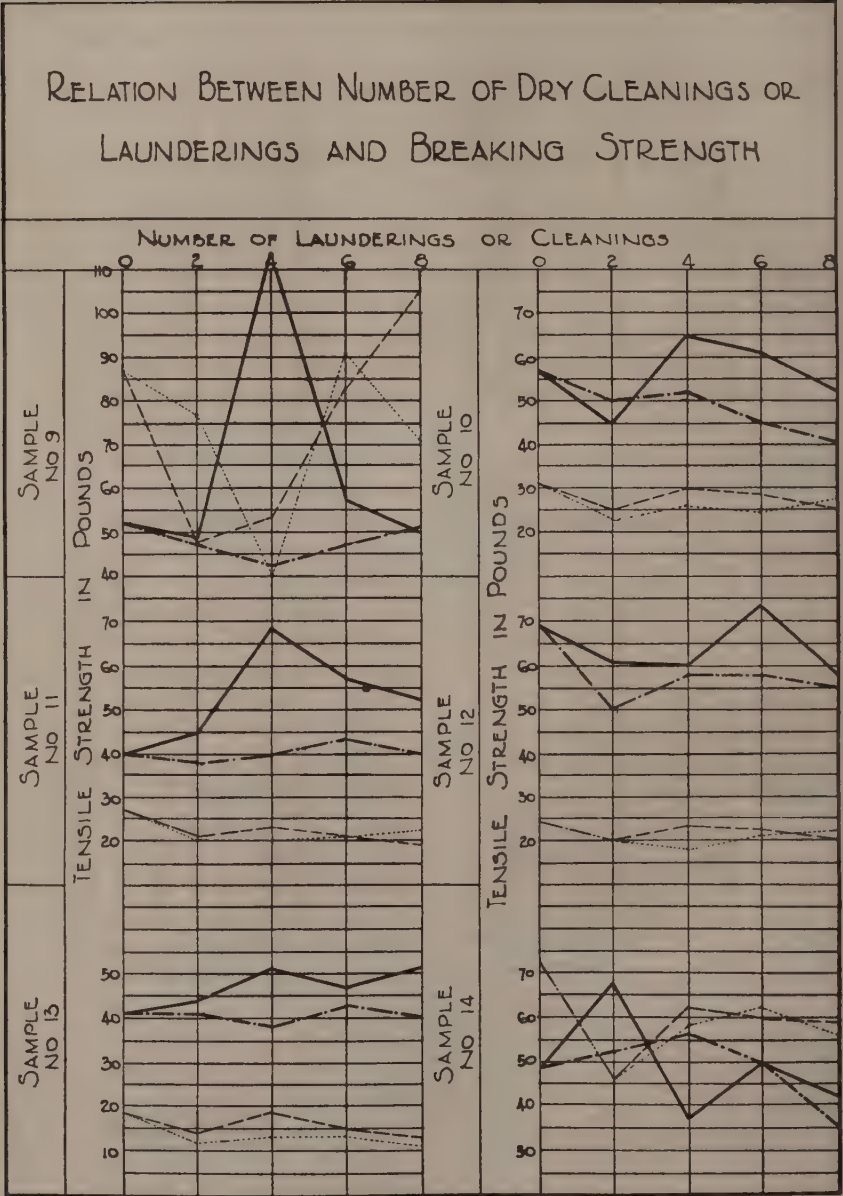


FIG. 4
(Problem I)

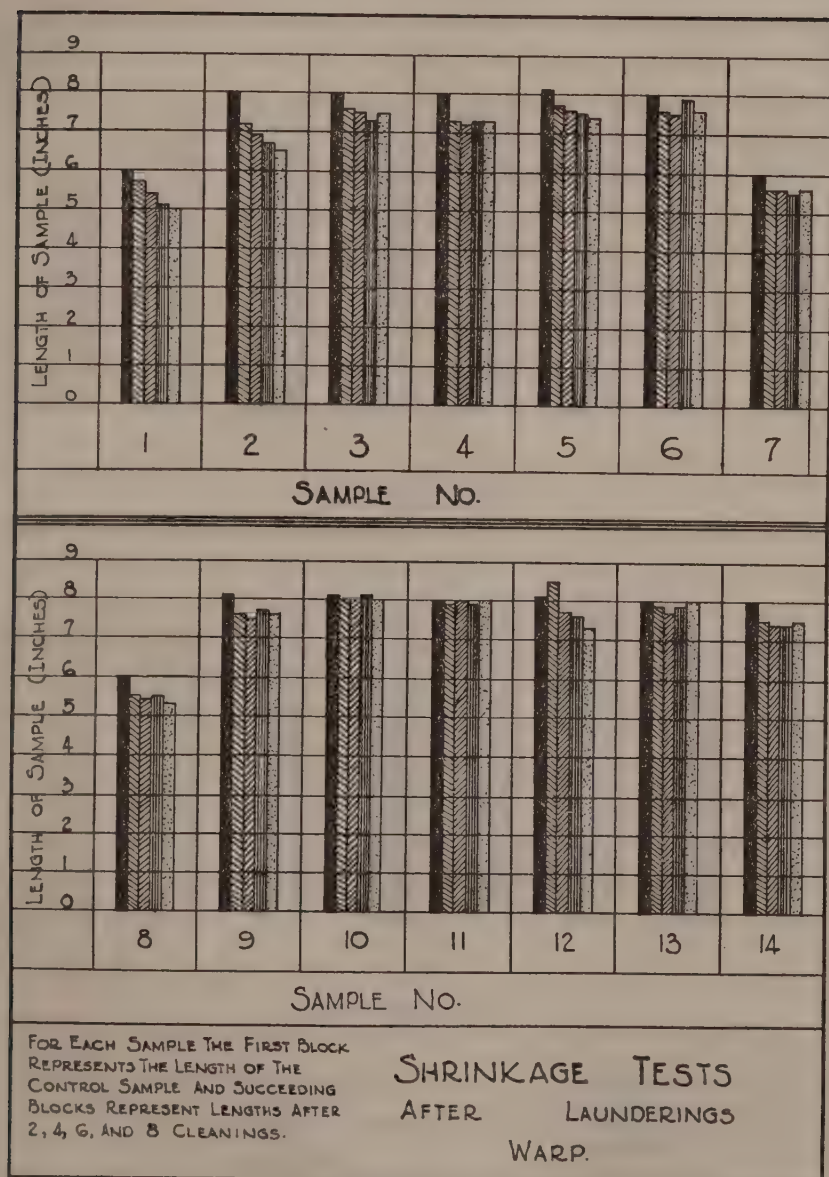


FIG. 5
(Problem I)

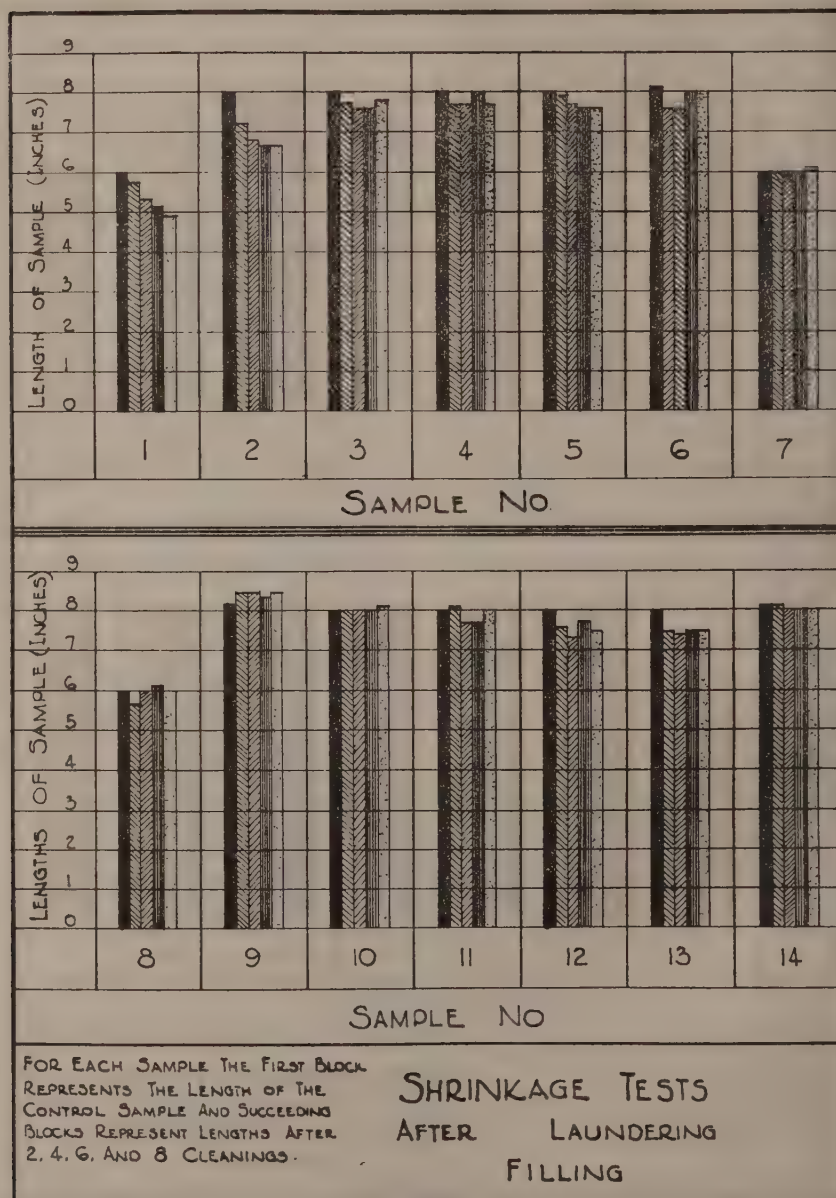


FIG. 6
(Problem I)

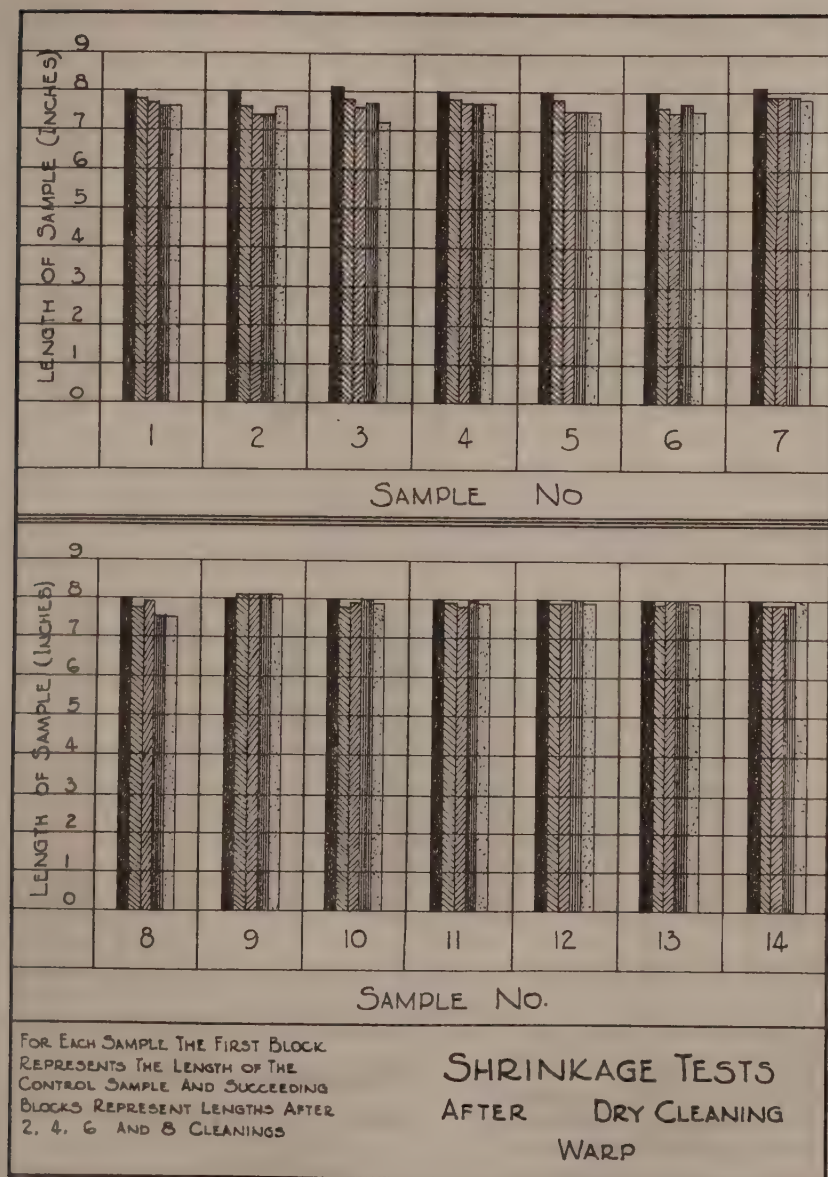


FIG. 7
(Problem I)

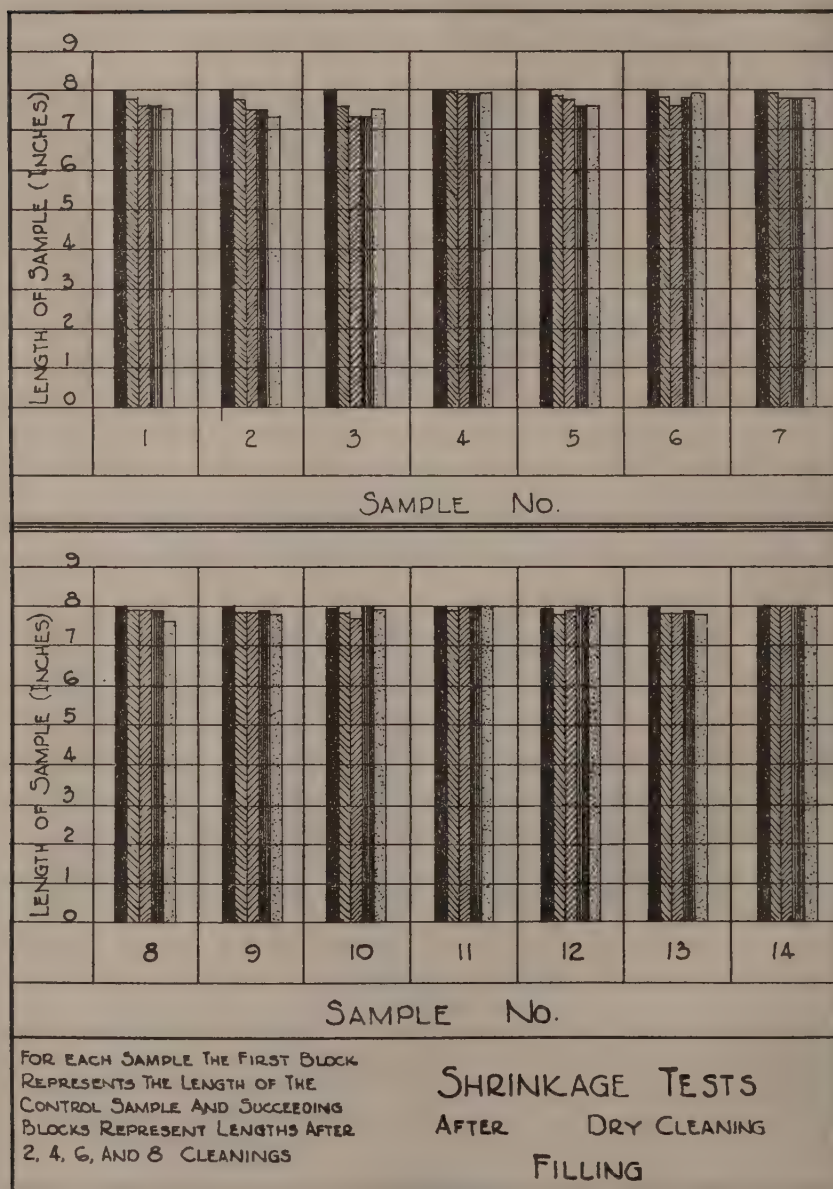


FIG. 8
(Problem I)

TABLE 2. *Elongation of seamed fabrics. Temperature 70° F. Relative humidity 65 per cent (Problem I)*

| Sample number | Control | | | | Seamed fabrics | | | | Deviation from control | |
|---------------|-----------------|-----|----|----|-----------------|-----|----|----|------------------------|-------|
| | Inch percentage | | | | Inch percentage | | | | Percentage | |
| | W | F | W | F | W | F | W | F | W | F |
| 1 | 0.9 | 0.9 | 29 | 31 | 0.6 | 0.9 | 25 | 30 | 33 | 0 |
| 2 | 0.8 | 0.9 | 26 | 38 | 0.9 | 1.3 | 31 | 44 | 12 | 44 |
| 3 | 1.4 | 1.1 | 46 | 54 | 1.5 | 1.4 | 52 | 48 | +71.4 | +27.2 |
| 4 | 1.2 | 1.6 | 27 | 39 | 1.0 | 1.3 | 34 | 44 | -16.7 | -18.8 |
| 5 | 0.9 | 0.8 | 29 | 26 | 0.9 | 1.0 | 38 | 34 | 0 | +25.0 |
| 6 | 1.1 | 0.8 | 38 | 28 | 1.3 | 0.4 | 44 | 21 | +18.1 | -50.0 |
| 7 | 0.7 | 1.0 | 24 | 35 | 1.0 | 1.2 | 35 | 42 | +42.8 | +20.0 |
| 8 | 0.8 | 1.2 | 28 | 41 | 1.0 | 1.3 | 40 | 43 | +25.0 | + 8.3 |
| 9 | 0.9 | 1.0 | 12 | 32 | 0.6 | 1.4 | 20 | 46 | -33.3 | +40.0 |
| 10 | 1.0 | 1.2 | 34 | 41 | 1.3 | 1.4 | 41 | 45 | +30.3 | +16.7 |
| 11 | 1.0 | 1.1 | 38 | 38 | 1.7 | 1.2 | 21 | 39 | -30.0 | + 9.0 |
| 12 | 1.2 | 1.2 | 40 | 41 | 1.0 | 0.6 | 43 | 21 | -83.3 | -50.0 |
| 13 | 0.7 | 0.7 | 24 | 22 | 0.7 | 0.8 | 25 | 37 | 0 | 0 |
| 14 | 0.7 | 0.6 | 23 | 20 | 0.8 | 0.8 | 27 | 26 | +14.3 | +33.3 |

TABLE 3. *Breaking strength of seam stitched fabrics. Temperature 70° F. Relative humidity 65 per cent (Problem I)*

| Sample number | Control lbs. | | Seam stitched fabric lbs. | | Deviation from control Percentage | |
|---------------|--------------|----|---------------------------|----|-----------------------------------|-------|
| | W | F | W | F | W | F |
| 1 | 19 | 21 | 16 | 12 | -16 | -42.8 |
| 2 | 15 | 9 | 12 | 6 | -20 | -30 |
| 3 | 24 | 22 | 18 | 16 | -40 | -36.6 |
| 4 | 18 | 15 | 18 | 14 | 0 | - .55 |
| 5 | 20 | 16 | 15 | 11 | -25 | -31 |
| 6 | 18 | 17 | 17 | 6 | -11 | -65 |
| 7 | 34 | 17 | 33 | 16 | - 2.9 | - 5.8 |
| 8 | 18 | 13 | 15 | 11 | -16.6 | -27.3 |
| 9 | 52 | 87 | 40 | 81 | -23 | -23.7 |
| 10 | 54 | 31 | 59 | 24 | + 9.2 | -22.5 |
| 11 | 40 | 27 | 32 | 21 | -20 | -22.2 |
| 12 | 69 | 24 | 56 | 21 | +19.9 | -12.5 |
| 13 | 41 | 18 | 21 | 15 | -48.7 | -16.6 |
| 14 | 49 | 72 | 55 | 66 | +12.2 | - 8.3 |

TABLE 1. *Analysis of fabrics.* (Problem II. Elongation and slippage of yarns in certain weighted silks)

| Fabrics | Yarn | | | | | | Folding resistance | |
|--------------------------------------|-----------------|----------------------|-------|-----------|-----------------|------------------------|----------------------------|----------------------|
| | Number per inch | Percentage of fabric | Count | | Twist | | Double fold per cm. fabric | Percentage deviation |
| | | | Typ | Direction | Number per inch | Per-cent-age deviation | | |
| A Degummed silk crêpe | W 83.0 | 64.9 | 123.7 | 2L, 2R | 0 | 1 | 2473 | 5 |
| | F 66.0 | 34.5 | 51.0 | | 57 | | 2267 | 7 |
| B Iron-weighted black silk crêpe | W 83.0 | 68.6 | 51.6 | 2L, 2R | 0 | 8 | 2274 | 7 |
| | F 66.4 | 31.0 | 40.4 | | 58 | | 9601 | 10 |
| C Lead-weighted white silk crêpe | W 64.4 | 62.7 | 37.2 | 2L, 2R | 0 | 8 | 563 | 11 |
| | F 71.0 | 36.4 | 38.0 | | 71 | | 2112 | 17 |
| D Tin-weighted white silk crêpe | W 90.0 | 65.3 | 47.3 | 2L, 2R | 0 | 3 | 469 | 3 |
| | F 76.0 | 34.1 | 41.2 | | 76 | | 2518 | 9 |
| E Tin-lead-weighted white silk crêpe | W 92.4 | 71.4 | 45.4 | 2L, 2R | 0 | 5 | 804 | 13 |
| | F 78.8 | 28.5 | 48.5 | | 84 | | 4383 | 14 |
| F Zinc-weighted white silk crêpe | W 78.0 | 66.9 | 53.5 | 2L, 2R | 0 | 3 | 813 | 5 |
| | F 70.0 | 33.1 | 41.6 | | 64 | | 3705 | 4 |

Note: W, F, L, R have been used as abbreviations for warp, filling, left, right. Fabric analysis run by Jeanette Ross, see Journ. H. Econ., 27:106-110. 1935.

TABLE 1. (Continued) *Analysis of fabrics (Problem II)*

| Percentages of | | | | | | | | | | | |
|---|------------------------|-------------------------------------|---------------|----------------|------|--------|------|------|-----|------|------------------|
| Fabric | Thick- ness Inch | Weight Ounces per sq. yard | Nitro- gen | Weight- ing | Ash | Silica | Iron | Lead | Tin | Zinc | Water extract |
| A Degummed silk crêpe | 0.0082 | 1.91 | 18.58 | | 0.23 | | | | | | |
| B Iron-weighted black silk crêpe | 0.0065 | 2.91 | 8.08 | 63.5 | 48.2 | 26.2 | 11.7 | | | | 11.3 |
| C Lead-weighted white silk crêpe | 0.0063 | 2.94 | 9.34 | 56.8 | 43.0 | 26.8 | | 11.3 | | | 6.9 |
| D Tin-weighted white silk crêpe | 0.0062 | 2.42 | 8.82 | 57.7 | 50.6 | 25.6 | | | 6.0 | | 4.4 |
| E Tin-lead-weighted white silk crêpe | 0.0065 | 3.15 | 8.44 | 60.8 | 50.3 | 27.6 | | 9.0 | 5.1 | | 4.4 |
| F Zinc-weighted white silk crêpe | 0.0067 | 2.87 | 7.89 | 58.9 | 46.3 | 23.4 | | | | 6.3 | 11.0 |

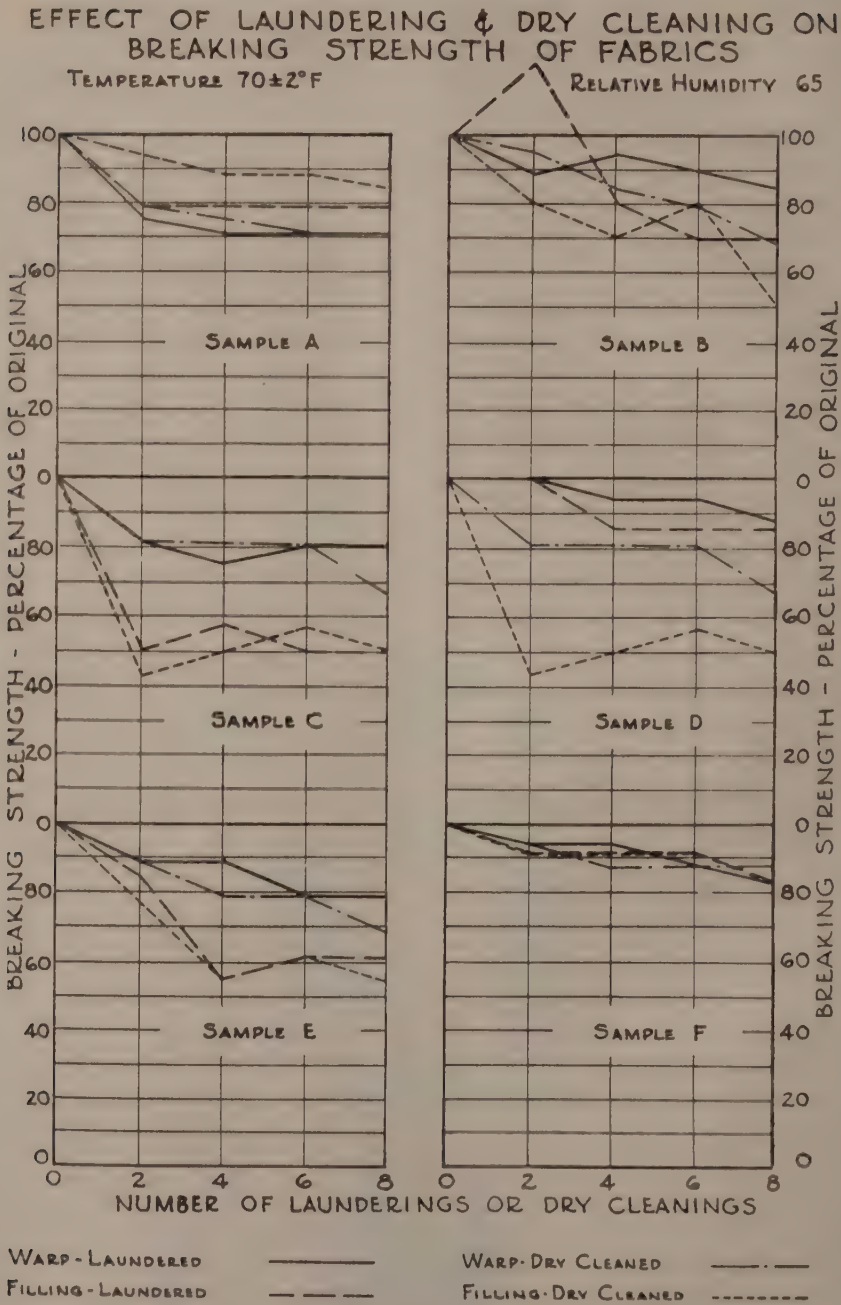
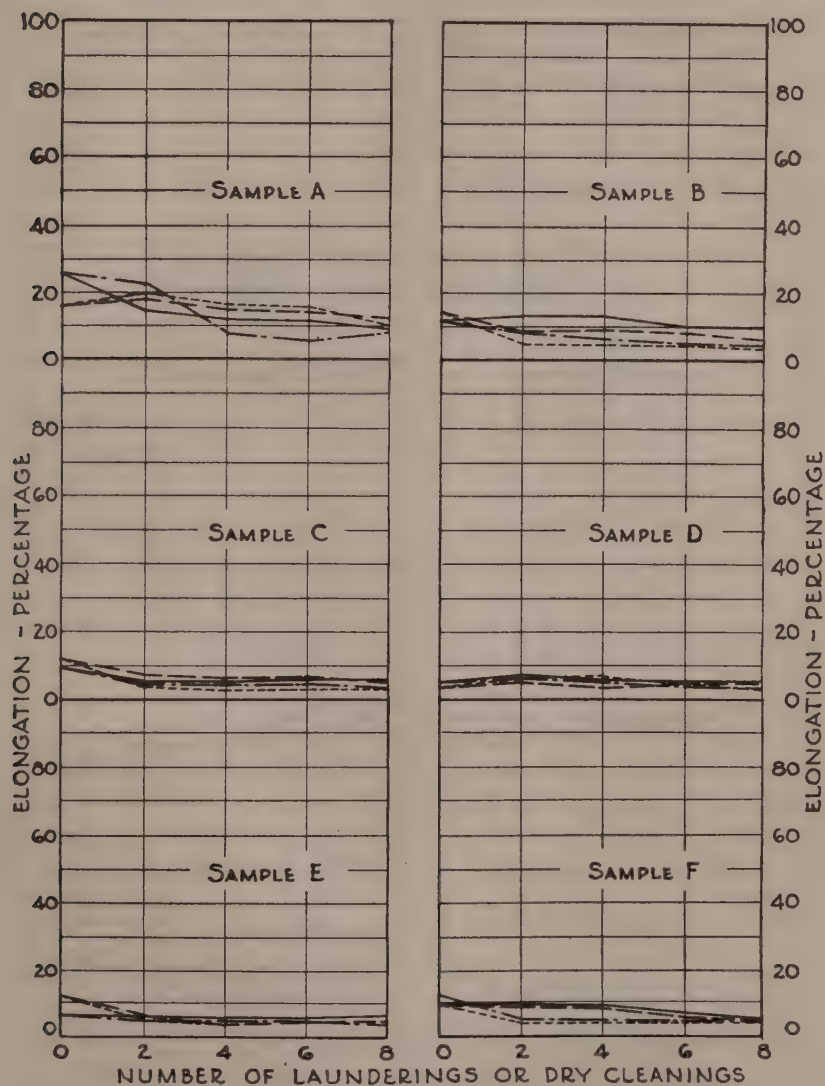


Fig. 1. (Problem II)

EFFECT OF LAUNDERING & DRY CLEANING ON
ELONGATION OF FABRICSTEMPERATURE $70 \pm 2^\circ\text{F}$.

RELATIVE HUMIDITY 65



WARP - LAUNDERED

————

FILLING - LAUNDERED

WARP - DRY CLEANED

- - - - -

FILLING - DRY CLEANED

- . - . -

Fig. 2. (Problem II)

EFFECT OF LAUNDERING & DRY CLEANING ON SHRINKAGE OF FABRICS

TEMPERATURE $70 \pm 2^\circ\text{F}$

RELATIVE HUMIDITY 65

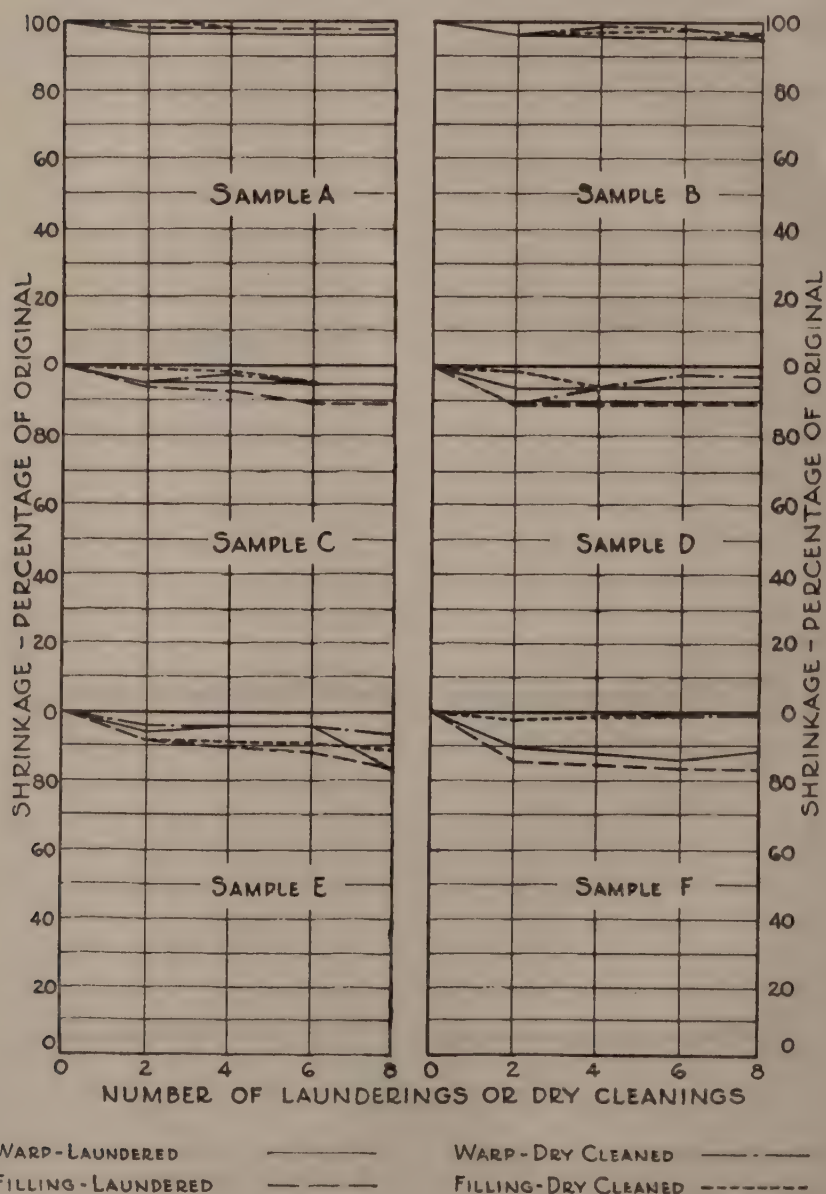


Fig. 3. (Problem II)

TABLE 2. *Effect of laundering and dry cleaning on breaking strength of seamed fabrics. Temperature 70° ± 2 F. Relative humidity 65 (Problem II)*

| After 10 launderings | | | | | | | | |
|--------------------------------------|------------------|----|------------------------|------|------------------------|------|------------------------|------|
| Fabrics | Original fabrics | | Plain seams | | French seams | | Flat fell seams | |
| | Lbs. per inch | | Percentage of original | | Percentage of original | | Percentage of original | |
| | W | F | W | F | W | F | W | F |
| A Degummed silk crêpe | 24 | 19 | 58.3 | 47.4 | 50.0 | 65.4 | 50.0 | 66.7 |
| B Iron-weighted black silk crêpe | 19 | 10 | 57.9 | 40.0 | 52.6 | 30.0 | 52.6 | 30.0 |
| C Lead-weighted white silk crêpe | 21 | 14 | 19.0 | 14.3 | 14.3 | 21.7 | 14.3 | 24.4 |
| D Tin-weighted white silk crêpe | 17 | 7 | 11.8 | 14.4 | 11.8 | 14.4 | 11.8 | 14.4 |
| E Tin-lead-weighted white silk crêpe | 19 | 13 | 15.8 | 7.7 | 21.1 | 23.0 | 12.8 | 23.0 |
| F Zinc-weighted white silk crêpe | 17 | 12 | 47.1 | 41.7 | 52.9 | 33.3 | 52.9 | 41.7 |

After 10 dry cleanings

| Fabrics | Original fabrics | | Plain seams | | French seams | | Flat fell seams | |
|--------------------------------------|------------------|----|------------------------|------|------------------------|------|------------------------|------|
| | Lbs. per inch | | Percentage of original | | Percentage of original | | Percentage of original | |
| | W | F | W | F | W | F | W | F |
| A Degummed silk crêpe | 24 | 19 | 60.5 | 52.6 | 50.0 | 52.6 | 60.0 | 73.7 |
| B Iron-weighted black silk crêpe | 19 | 10 | 52.6 | 20.0 | 26.3 | 30.0 | 31.7 | 26.0 |
| C Lead-weighted white silk crêpe | 21 | 14 | 33.3 | 14.3 | 22.9 | 21.4 | 23.8 | 21.4 |
| D Tin-weighted white silk crêpe | 17 | 7 | 5.9 | .0 | 11.8 | .0 | 5.9 | .0 |
| E Tin-lead-weighted white silk crêpe | 19 | 13 | 5.3 | .0 | 5.3 | .0 | 5.3 | .0 |
| F Zinc-weighted white silk crêpe | 17 | 12 | 37.1 | 33.3 | 29.4 | 41.7 | 35.3 | 35.0 |

Note: The size of the needle used in the stitching was 00 and the thread was silk, A. There were sixteen stitches to the inch.

TABLE 3. *Effect of laundering and dry cleaning on elongation of seamed fabrics. Temperature 70° ± 2 F. Relative humidity 65 (Problem II)*

Elongation at breaking load after 10 launderings

| Fabrics | Plain seams | | French seams | | Flat fell seams | |
|--------------------------------------|---------------------------|------|---------------------------|------|---------------------------|-----|
| | Per-centage of elongation | | Per-centage of elongation | | Per-centage of elongation | |
| | W | F | W | F | W | F |
| A Degummed silk crêpe | 8.0 | 12.0 | 10.0 | 12.3 | 9.0 | 8.0 |
| B Iron-weighted black silk crêpe | 5.0 | 5.7 | 5.3 | 6.7 | 5.3 | 5.3 |
| C Lead-weighted white silk crêpe | 3.7 | 2.3 | 2.7 | 4.0 | 2.7 | 4.0 |
| D Tin-weighted white silk crêpe | 1.0 | 0.3 | 1.3 | 0.3 | 1.0 | 0.3 |
| E Tin-lead-wieghted white silk crêpe | 1.0 | 0.3 | 1.3 | 0.3 | 1.0 | 0.3 |
| F Zinc-weighted white silk crêpe | 6.0 | 5.5 | 4.0 | 5.0 | 4.0 | 3.0 |

Elongation at breaking load after 10 dry cleanings

| Fabrics | Plain seams | | French seams | | Flat fell seams | |
|--------------------------------------|---------------------------|------|---------------------------|------|---------------------------|------|
| | Per-centage of elongation | | Per-centage of elongation | | Per-centage of elongation | |
| | W | F | W | F | W | F |
| A Degummed silk crêpe | 12.7 | 10.0 | 13.3 | 16.7 | 12.0 | 10.7 |
| B Iron-weighted black silk crêpe | 5.3 | 6.0 | 5.3 | 4.0 | 5.0 | 4.0 |
| C Lead-weighted white silk crêpe | 4.3 | 2.0 | 4.3 | 2.0 | 3.7 | 2.3 |
| D Tin-weighted white silk crepe | 0 | 0 | 0 | 0 | 0 | 0 |
| E Tin-lead-weighted white silk crêpe | 0 | 0 | 0 | 0 | 0 | 0 |
| F Zinc-weighted white silk crêpe | 4.7 | 3.3 | 4.0 | 3.7 | 3.0 | 4.0 |

The results indicated that the zinc-weighted silk crêpe combined a greater number of qualities which make for durability than any of the fabrics tested. It had a medium-spun yarn, was well balanced as to yarns, had little shrinkage and little loss in breaking strength. These results agree with Johnson's (3) statement concerning the twist and shrinkage of yarns.

There was no indication from the data obtained that the amount of weighting affected elongation.

Lack of balance of yarns in a fabric, a small number of yarns to the inch, great variation in yarn count, small number of yarn twists per inch, and seaming of fabrics (when pressure was applied) appeared to increase slippage of yarns (Plate I) and elongation in the fabrics tested.

Laundering and dry cleaning affected elongation in all of the fabrics tested. Definite conclusions are not justified because of the variable results of the experiments, as shown in figures 1 and 2 of Problem I and figure I in Problem II.

In Problem I there was greater shrinkage in the greater proportion of the fabrics from laundering than from dry cleaning. The amount of

shrinkage in the weighted silks was approximately equal in the two cleaning processes. The greater proportion of the total shrinkage in the woolen fabrics occurred during the first two launderings. In general, there was greater shrinkage from cleaning in the woolen fabrics than in the silk fabrics. Rajah silk showed practically no shrinkage after dry cleaning. During the cleaning processes, some of the fabrics stretched while others shrank. Variation in stretch and shrinkage were affected to some extent by the tension at which the fabric was held during the pressing.

The yarns in loosely woven fabrics slipped or the fabrics broke at the seam when strained at right angles to the direction of a seam. The stitching in the stronger fabrics gave way before the fabrics broke. The yarns in both weighted and unweighted flat crêpes slipped at the seams readily. These fabrics had fine filling yarns and no twist in the warp. The twill weave silk fabrics, which were stronger, broke above or below the seam. The fabrics which had the flat-fell seams registered the highest breaking strength and the lowest elongation.

The silk fabrics which stood up well under the tests were the lead, the tin, and the tin-lead-weighted silk crêpes. These were high in yarn count, lost little from shrinkage, were low in original percentage of elongation, and average in breaking strength, after both the laundering and dry cleaning, as shown in table 1 of Problem II.

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PLATE I

Slippage of yarns.

PLATE I



ROLE OF PHOSPHOGLYCERIC ACID IN THE DISSIMILATION OF GLUCOSE BY THE PROPIONIC ACID BACTERIA

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Accepted for publication March 24, 1936

The recent work of Embden, Deuticke and Kraft (1933) and of Meyerhof and Kiessling (1933) on muscle glycolysis relegates the methylglyoxal scheme of glucose breakdown to a relatively unimportant position. These investigators propose the phosphoglyceric acid mechanism to account for the dissimilation of glucose wherein methylglyoxal does not occur and lactic acid is not formed as a stabilization product. Instead, phosphoglyceric acid is the intermediary which yields pyruvic acid. The latter is then hydrogenated to form lactic acid.

In view of the general acceptance of the methylglyoxal scheme in bacterial dissimilation, it is of interest and importance to know that phosphoglyceric acid has been isolated as an intermediary from the products of dissimilation of glucose by the propionic acid bacteria (*Propionibacterium*). The evidence indicates that the importance of methylglyoxal in bacterial dissimilation must be re-evaluated in the light of our present knowledge of the occurrence of phosphoglyceric acid.

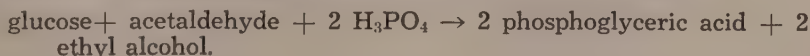
Phosphoglyceric acid was isolated from the cultures of three distinct species, *Propionibacterium shermanii*, *P. arabinosum* and *P. pentosaceum*.

The method of Vercellone and Neuberg (1935) was followed with modification. The bacteria were grown in a glucose-yeast extract broth at 30° C. The cells were centrifuged from the medium after three days' incubation. To a small flask were added: cells (paste) 7.0 gm.; 0.66M phosphate buffer, 7.0 cc.; 20 per cent glucose solution, 5.6 cc.; 2.5 per cent sodium hexose-diphosphate solution, 5.6 cc.; 2 per cent acetaldehyde, 8 cc.; 1 per cent MgCl₂, 0.2 cc.; 0.2M sodium fluoride solution, 1.5 cc.; and toluene, 1 cc.

The mixture was incubated for 6 hours at 37° C. with frequent shaking, then chilled, and placed in a refrigerator. Within 36 hours the mixture was treated according to Vercellone and Neuberg and the phosphoglyceric acid present was precipitated as the barium salt. In the following table are the results for the three species employed.

| Species | Yield of phosphoglyceric acid (barium salt) mgm. |
|------------------------------|--|
| <i>P. arabinosum</i> (34 W) | 116 |
| <i>P. pentosaceum</i> (49 W) | 169 |
| <i>P. shermanii</i> (52 W). | 30 |

According to Meyerhof and Kiessling (1934) the phosphoglyceric acid arises by the following reaction:



The hexose diphosphate is a catalyst and the sodium fluoride prevents dissimilation of phosphoglyceric acid to pyruvic acid. In a normal propionic fermentation the pyruvic acid is reduced, first to lactic and then to propionic acid. It is probable that acetaldehyde does not occur as a normal intermediary, although when added it is reduced in part to ethyl alcohol.

It is of value to know whether propionic acid bacteria are able to form phosphoglyceric acid from glucose in the absence of hexose-diphosphate. In this case the evidence would further support the Embden-Meyerhoff scheme. It was necessary to provide suitable conditions for the phosphorylation of glucose before adding the acetaldehyde and fluoride (Neuberg and Kobel, 1933). The following experiment was run, using *P. pentosaceum*:

In a 300 cc. flask were placed 23 g. of cell paste, diluted to 50 cc. with distilled H_2O ; 50 cc. 25 per cent glucose; 50 cc. 0.66 M phosphate buffer (pH 6.95); and 10 cc. of 0.2 M sodium fluoride. The mixture was incubated at 30° C for 24 hours. During this period there was an uptake of 0.94 mg. of inorganic phosphate per cubic centimeter (calculated as P). The phosphate content was determined colorimetrically by the method of Kuttner and Lichtenstein (1930). After 24 hours an additional 10 cc. of fluoride solution and 25 cc. of 2 per cent acetaldehyde were added and the mixture incubated for 4 hours. During this period 0.14 mg. P per cubic centimeter was esterified. From the mixture 0.417 gm. of the crystalline barium salt of phosphoglyceric acid was obtained.

The crude phosphoglyceric acid salt on purification yielded a pure white product which crystallized from an alcohol-water solution in white leaflets possessing the same distinctive sheen as the product prepared from yeast. The optical rotation, $[\alpha]_D^{23} C. = -13.0$ is very close to that obtained by Neuberg and Kobel (1933).

It may be objected that the formation of phosphoglyceric acid by bacteria in the presence of toluene does not prove the acid to be a normal intermediary in the dissimilation of glucose; however, its isolation does indicate that the propionic acid bacteria at least possess the necessary enzyme equipment for its formation. Furthermore, the evidence is clear that the breakdown of glucose may occur by way of phosphoglyceric acid. In addition it appears that the mass of evidence supporting the Embden-Meyerhof scheme of glycolysis may be equally well applied now to bacteria.

In a later communication the principles of the Embden-Meyerhof scheme will be applied to the dissimilation by the propionic acid bacteria.

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ECONOMIC STUDIES OF SCREW WORM FLIES, *COCHLIOMYIA* SPECIES (DIPTERA, CALLIPHORINAE), WITH SPECIAL REFERENCE TO THE PREVENTION OF MYIASIS OF DOMESTIC ANIMALS¹

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INTRODUCTION

Heavy losses are suffered each year by the various livestock interests throughout the southwestern part of the United States owing to the destructive activities of the screw worm flies, *Cochliomyia americana* C. & P. and *C. macellaria* Fab. Babcock and Bennet (1) estimated this damage at \$4,000,000 annually. Parman (8) states that the loss in some years has been estimated at \$5,000,000, and according to Laake and Cushing (7) southwestern ranchmen estimated the losses in 1928 due to the screw worm and fleece worm at \$10,000,000.

Screw worm flies are strongly attracted to the slightest wound or diseased tissue of an animal. Either the living or the necrotic tissues of the host are favorable sites for the deposition of eggs. The feeding of the resulting larvae causes a rapid destruction of the tissues involved and very often death within a few days if treatment is not promptly administered.

Parman (8) reports the results of extensive experiments with larvicides for killing the larvae in wounds of animals. Bishopp et al. (2), Parman et al. (9) (10), and Laake et al. (6) studied the chemotropic responses of screw worm flies to numerous chemicals with the object of finding a material suitable for wound application and of a strong and lasting repellent power for the protection of the wound from reinfestation. Their studies have yielded valuable information on suitable materials for the destruction of the larvae in the wound and the protection of the wound from reinfestation for a reasonable length of time. Bishopp et al. (3) also point out the necessity of range sanitation to prevent the breeding of myiasis-producing species of flies and suggest modifications in the methods of range management in order to reduce myiasis during the screw worm season.

No quantitative data on the predisposing causes of myiasis or the feasibility of controlling myiasis-producing flies by large scale, systematic

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The writer is greatly indebted to E. C. Cushing, Senior Entomologist, and H. E. Parish, Assistant Entomologist, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, for collecting much of the data presented herein. To over 75 ranchmen of Menard County Texas, he is indebted also, for the thousands of case records which they supplied. Without their wholehearted cooperation this investigation would not have been possible.

trapping have been reported. Considering the great economic importance of screw worms and the lack of reliable information on these phases of the problem, it was evident that a more extensive study was urgently needed.

During the screw worm seasons of 1929 to 1933, inclusive, a rather extensive study of the predisposing causes of myiasis in domestic animals was conducted; during 1931 to 1933, inclusive, the effect of systematic trapping on the incidence of myiasis was investigated. Studies designed to test the efficiency of several types of blowfly traps and to determine desirable trap locations also were conducted during the seasons of 1929 to 1933, inclusive, but those data will appear elsewhere.

METHODS, MATERIALS, AND DESCRIPTION OF THE AREA INVOLVED

In the study designed to determine the predisposing causes which render animals susceptible to attack by screw worm flies, more than 75 ranches located in Menard, Kimble, and Schleicher Counties, Texas, constituted the locality in which this investigation was made. Each ranch operator was provided with printed forms on which to record new cases of myiasis. The data recorded on each case consisted of the date, class of animal infested, and predisposing cause of the infestation. The injury sustained or the condition responsible for each new case was determined as nearly accurately as possible by the ranchman. The age of the animal and the methods locally employed for the handling of animals of different ages constituted the basis for the grouping of animals of the same or closely related species into different classes.

The flytraps used were those generally known as the "Government, all-metal, cone type" described by Bishopp (4). Gray enameled bait pans, three and three-fourths to four inches deep by 13 inches in diameter at the top, with a bait capacity of approximately 6 quarts, were used. The bait consisted of fresh goat or sheep meat and water. Nicotine sulphate at the rate of 3 to 4 cc. per gallon of water was added to the bait to prevent the development of larvae in the bait pans. The amount of bait used for each trap and the baiting schedule adopted for each of the three seasons were as follows: Two pounds of meat and approximately 6 quarts of water containing the nicotine sulphate were placed in each bait pan at the beginning of the season. The pans were cleaned and rebaited at 14- to 26-day intervals, depending upon temperature and rate of evaporation. One pound of meat and the amount of evaporated water were added on the seventh to thirteenth day, or about the middle of the bait-exposure period. No nicotine sulphate was added to the water used for the refill. The rate of decomposition of the meat and evaporation of the water is greatly accelerated by the heat during the summer months or during extended periods of continuous high winds. It is obvious, therefore, that to secure the greatest efficiency from the trap throughout the variable climatic seasons of screw worm activity no predetermined schedule could be followed. For most of the trapping period, however, it was found necessary to adhere to the shorter of the rebaiting schedules indicated above.

Each trap was placed on a triangular wooden platform nailed approximately 4 feet above the ground between two trees. The legs of the trap were fastened to the platform to avoid its being disturbed by animals or high winds. The locations were carefully selected, and the trap platforms were made level and fastened to trees of sufficient size to avoid any

considerable amount of swaying during high winds with consequent spilling of the bait.

The area on which traps were used consisted of 154,879 acres in 1931, 155,679 acres in 1932, and 152,479 acres in 1933. This area is in the Edwards Plateau region and is comprised of typical west Texas ranch land in the northwestern part of Menard County, Texas, along the north side of the San Saba River, which properly begins in springs in the vicinity of Fort McKavett, situated at the southwestern corner of this area. The elevation ranges from 1,950 to about 2,200 feet above sea level. The rainfall averaged approximately 22.8 inches per year for 6 years, according to unofficial local records. The topography ranges from gently rolling and hilly to moderately rough and broken land. The ridges and often the hillsides throughout this area are very stony. There are few running streams other than the San Saba River, but numerous shallow, dry creeks and draws separate the hills. The vegetation consists of clustered and scattered live oak, shin oak, and mesquite trees of small to medium size, and various small, thorny shrubs. Various weeds and grasses grow luxuriantly along the draws and hillsides when rainfall is sufficient. Buffalo grass, a turf grass, is the principal forage plant for cattle and sheep in this area. The non-grass-like plants that also serve for forage are largely milk vetches (*Astragalus*), plantains (*Plantago*), tallow-weed (*Amblyolepis*), and daisies (*Aphanostephus*). Late in the winter and early in the spring certain annual grasses, such as fescue, a species of *Trisetum*, and rescue grass, form the important forage plants. There is little cultivation, less than one per cent, in the area in question.

Six hundred and sixty-four traps were rather uniformly distributed over the test area at an average spacing of one trap for approximately every 232 acres, or nearly 2.76 traps per section based on the 1932 and 1933 average. The traps were operated from March 1 to October 31 of each of the three years. Approximately every 21 days all the dead flies that had accumulated in the traps were removed and measured.

The control or untrapped area consisted of 144,860 acres in 1931, 172,000 acres in 1932, and 113,580 acres in 1933. The ranches in this area were somewhat scattered and were situated in the southern portion of Menard County, in the northern portion of Kimble County, and in the west central portion of Schleicher County. The elevation, topography, vegetation, precipitation, etc., in the localities in which the various ranches comprising the non-trapped area were located are very similar to those in the trapped area. Also, the management of the ranches and the seasonal range practices are very similar.

The animal population of every ranch was recorded at monthly intervals and averaged at the end of the season for each class of animal on each ranch in both areas.

All cases of myiasis in both areas were recorded on the cards described above. These records were collected from each ranch approximately every 6 to 8 days throughout the season. The person on each ranch detailed to the duty of determining the predisposing causes of myiasis and keeping the records was considered competent in most cases to perform these tasks. Occasionally, however, cases were not promptly recorded and it was, therefore, made a rule to question the record keeper weekly on each ranch to ascertain if all cases were recorded. If the case records

were not complete, a record for each of the cases missed was then made. The person questioned usually was able to give a complete history of the unrecorded cases, but in case of doubt as to the predisposing cause of the infestation or the class of animal infested the record was marked as questionable and was not included in the data dealing with the predisposing causes.

EXPERIMENTAL DATA

The experimental data are presented in two parts: (a) Predisposing causes of myiasis; (b) systematic trapping of screw worm flies.

PREDISPOSING CAUSES OF MYIASIS

The purpose of this investigation was to determine the predisposing causes of myiasis and their relative importance in seven classes of domestic animals. These data should be useful as a basis for recommending such modifications of range practices as would result in the reduction of the number of screw worm cases. The general data for the field of the experiments are given in table 1.

TABLE 1. *Number of ranches and of acres, average number of animals, and number of screw worm cases, 1929-1933*

| Year | Number of ranches | Number of acres | Average monthly animal population | Number of screw worm cases | Percentage of animals infested |
|------|-------------------|-----------------|-----------------------------------|----------------------------|--------------------------------|
| 1929 | 35 | 176,185 | 116,361 | 3,332 | 2.86 |
| 1930 | 34 | 167,879 | 104,386 | 1,442 | 1.38 |
| 1931 | 70 | 299,739 | 187,050 | 4,055 | 2.17 |
| 1932 | 68 | 327,679 | 193,444 | 8,315 | 4.30 |
| 1933 | 72 | 266,059 | 194,978 | 4,094 | 2.10 |

In every case where the predisposing cause was uncertain, owing to the fact that the infested animal was not found until the infestation had spread and the destruction of the tissues had become so extensive that the nature of the predisposing cause was no longer evident, it was recorded as unknown. Such unknown predisposing causes are not included in table 2 or the graphs. Table 2 shows all the predisposing causes and the number of cases of myiasis due to each predisposing cause in the seven classes of animals, and figure 1 shows the principal predisposing causes and their relative importance as recorded during the period from March 1 to October 31 of the five years in question.

In view of the great number of cases recorded and the number of years during which the observations were made, it is probable that all the locally common and most of the unusual predisposing causes of myiasis in seven classes of animals as shown in table 2 have been established. It should be remembered, however, that since environments differ in various parts of the area affected by screw worm flies, predisposing causes other than those listed herein might be encountered. For example, in a limited area along the Gulf Coast it is a well-known fact that one of the most common predisposing causes of myiasis in several classes of animals is the

TABLE 2. *Predisposing causes of myiasis and incidence of each in seven classes of domestic animals*

| Predisposing causes | Causes of myiasis in classes of animals | | | | | | |
|---------------------------------|---|-------|-------|------|--------|--------|------------------|
| | Sheep | Lambs | Goats | Kids | Cattle | Calves | Horses and mules |
| | Number | | | | | | |
| Accidental injuries | 285 | 84 | 283 | 44 | 209 | 105 | 74 |
| Beggar lice (seed) ¹ | 21 | 6 | 17 | 3 | 0 | 0 | 0 |
| Birth | 116 | 110 | 17 | 49 | 209 | 2,769 | 20 |
| Boils | 44 | 1 | 442 | 4 | 13 | 19 | 1 |
| Branding | 0 | 0 | 4 | 0 | 9 | 26 | 1 |
| Broken horn | 19 | 3 | 44 | 3 | 9 | 4 | 0 |
| Broken leg | 0 | 1 | 5 | 0 | 0 | 0 | 0 |
| Brush scratches | 19 | 10 | 369 | 26 | 7 | 1 | 4 |
| Cancer eye | 0 | 0 | 0 | 0 | 67 | 3 | 0 |
| Castrating | 1 | 91 | 17 | 48 | 4 | 509 | 1 |
| Dehorning | 37 | 1 | 0 | 0 | 35 | 459 | 0 |
| Docking | 0 | 404 | 0 | 0 | 0 | 0 | 0 |
| Dog or hog bite | 5 | 1 | 6 | 4 | 4 | 0 | 0 |
| Fighting | 1,779 | 3 | 14 | 0 | 14 | 0 | 0 |
| Genitalia injuries | 3 | 0 | 0 | 0 | 6 | 0 | 37 |
| Hooked by cattle | 3 | 0 | 6 | 1 | 797 | 137 | 1 |
| Horn flies | 749 | 135 | 22 | 1 | 382 | 159 | 0 |
| Horse flies | 0 | 0 | 0 | 0 | 61 | 11 | 0 |
| Lice | 1 | 4 | 75 | 13 | 0 | 0 | 0 |
| Marking | 3 | 234 | 0 | 60 | 1 | 48 | 0 |
| Needle grass | 1,999 | 713 | 74 | 4 | 0 | 2 | 0 |
| Old sores | 7 | 0 | 2 | 0 | 2 | 1 | 0 |
| Ox warble | 0 | 0 | 0 | 0 | 6 | 0 | 0 |
| Pink eye | 0 | 1 | 0 | 0 | 6 | 1 | 0 |
| Prickly pear (cactus) | 153 | 9 | 20 | 0 | 1 | 0 | 0 |
| Rock-bruised feet | 9 | 0 | 1 | 0 | 0 | 0 | 1 |
| Rope burn | 0 | 0 | 0 | 0 | 1 | 1 | 9 |
| Saddle sores | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Scours | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| Shear cuts | 768 | 27 | 3,521 | 187 | 0 | 0 | 0 |
| Snagged | 4 | 0 | 1 | 0 | 8 | 0 | 6 |
| Snake bite | 0 | 0 | 0 | 0 | 3 | 2 | 1 |
| Sore mouth | 470 | 381 | 17 | 7 | 0 | 1 | 0 |
| Spaying | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Stud bites | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Ticks | 1 | 0 | 3 | 0 | 1 | 4 | 0 |
| Tooth decay | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| Udder injuries | 49 | 0 | 39 | 0 | 17 | 0 | 0 |
| Warts | 0 | 0 | 24 | 0 | 15 | 11 | 0 |
| Wire cuts | 7 | 0 | 19 | 0 | 73 | 29 | 264 |
| Total | 6,552 | 2,220 | 5,045 | 455 | 1,960 | 4,304 | 426 |

¹ *Daucus pusillus*.

injury produced by the Gulf Coast tick, *Amblyomma maculatum* Koch. This tick does not appear elsewhere.

The relative importance of the predisposing causes of myiasis also was well established for each of the seven classes of animals. This is

shown by the diagrams in figure 1. Since there are many local or sectional variations in the area affected by the screw worm flies, the most common predisposing causes will no doubt vary somewhat in different localities, but in view of the fact that most of the environments encountered in the test area are very similar to those existing over millions of acres of ranch land in the livestock areas of western and southwestern Texas, the results that were obtained in the area in question are applicable to most of the affected territory in the state.

The information obtained concerning the contributing factors responsible for the various predisposing causes indicates that it is possible to reduce greatly or eliminate some of these. In order to avoid confusion, it seems advisable to discuss separately the factors responsible for each of the various predisposing causes for each class of animal.

Sheep.—Over 30 per cent of all myiasis in sheep was reported as having been caused by needle grass (*Aristida*). The needle-shaped seeds of this grass become imbedded in the wool of the animal and irritate the tissues, with the result that the animal becomes susceptible to attack. Since abundant growth of needle-grass is usually associated with a light and tight type of soil, often brought about by over-stocking and subsequent erosion, it will always be a serious factor during years of abundant rainfall. It is a well-known fact, however, that timely and proper stocking of needle-grass pastures will greatly reduce the amount of seed formed and somewhat reduce the myiasis incidence due to it. It is known also that shearing the wool from the face and lower parts of the legs, where most of the needles become imbedded, will result in a marked decrease of myiasis from this source. It is claimed that the loss in the prematurely clipped wool and the labor involved is less than that occasioned by the injuries from screw worms and from the labor and material required for treating and caring for the infested animal.

Fighting, responsible for 27 per cent of all myiasis in this class, occurs mostly among bucks, and the wounds so sustained are confined to the head. Dehorning has not relieved the situation, but the breeding of hornless strains has possibilities. The separation of the habitual fighters from the rest of the bucks in the flock has helped somewhat, but has not proved practicable, according to ranchers who have tried this practice. No really practicable preventive methods are known. It would seem, therefore, that fighting among bucks is not a predisposing cause that can be reduced materially.

Shear cuts have been found to be responsible for over 11 per cent of all cases of myiasis among sheep. In sheep and goat raising sections of the affected area much of the shearing is done at a stipulated price per animal. In order to obtain the maximum daily wage, the shearing is done as rapidly as possible and usually with a disregard for minor shear cuts in the tissues of the animal. Furthermore, with a few exceptions, the injuries so sustained by the animal are not treated with a repellent to protect the wound from fly attack. It is obvious that even the best shearers will occasionally cut an animal and thereby predispose it to screw worm attack, but in the area in question it is quite certain that in the majority of cases the wounding of the animals is caused by the type of clipper used, the haste and carelessness of the operator, and the lack of an application of a good repellent to the wounds before the animals are liberated from the shearing

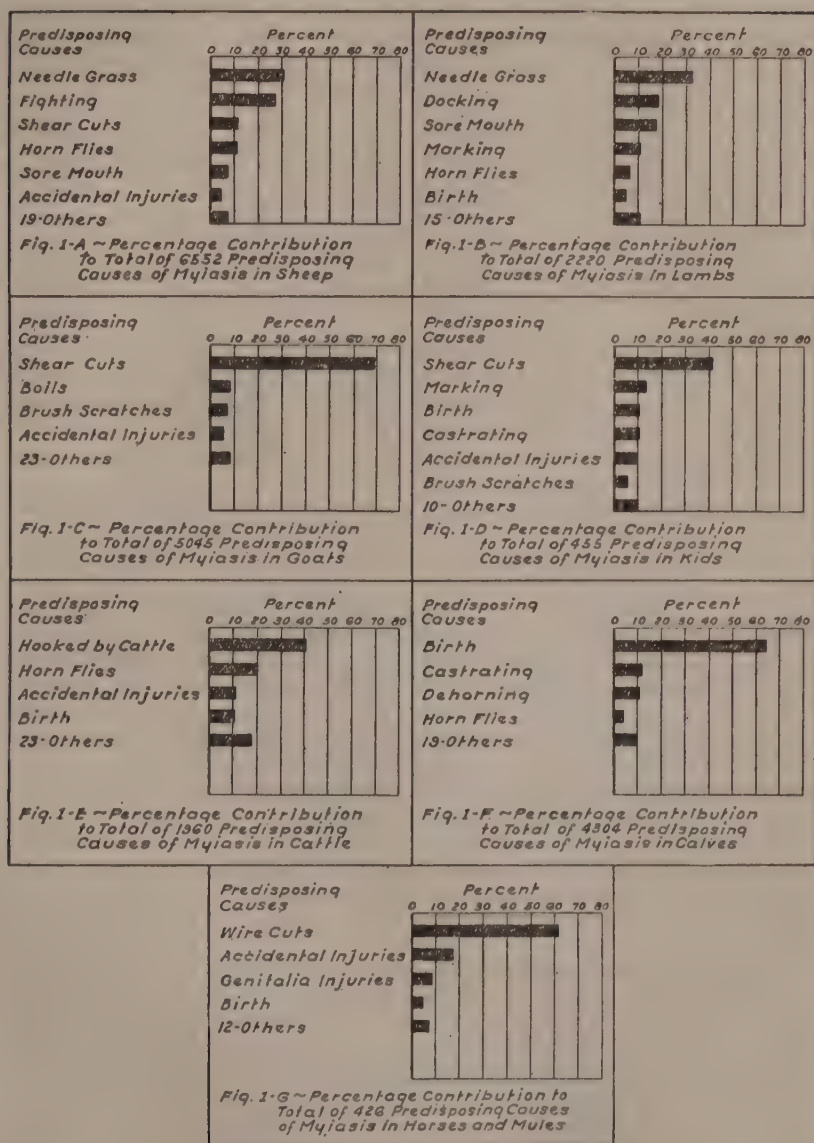


Fig. 1. Diagram showing percentage contribution to total principal predisposing causes of myiasis in seven classes of domestic animals in Menard County, Texas, 1929-1933.

pens. If these underlying factors are eliminated, it would seem reasonable to expect at least a 75 per cent reduction of myiasis due to shear cuts.

Horn flies (*Haematobia irritans* L.) are a predisposing cause for 11 per cent of myiasis cases in this class of animal. Methods commonly prac-

ticed for the control of this fly on dairy cattle are not practicable for sheep under range conditions. Feasible methods of control of the horn fly under range conditions are unknown; therefore, it is at present impossible to reduce screw worm cases arising from its injuries.

Sore mouth (contagious ecthyma), a disease of sheep and goats, is the predisposing cause of over 7 per cent of all cases of myiasis in this class. Vaccination for the control of this disease is feasible but not yet practiced except on badly infected ranches. The number of cases resulting from this predisposing cause is, therefore, subject to reduction.

Accidental injuries are responsible for approximately 5 per cent of all myiasis cases in sheep. Since man has little control over such injuries, the reduction of myiasis due to this predisposing cause is not likely to be realized.

Prickly-pear injuries are usually confined to relatively few animals, and, in most instances, are due to the eating of the fruit and the so-called leaves of the plant. The spines cause the injury which induces attack by screw worm flies. To eliminate this predisposing cause of myiasis the habitual cactus feeders, in regions where no cactus-free land is available, should be marketed.

Birth as a predisposing cause of myiasis is found only on poorly managed ranches where bucks are permitted to breed the ewes at a time when the resulting lambs drop during the screw worm season. This predisposing cause is preventable.

Among the less frequent predisposing causes of myiasis in sheep are the operations of dehorning, marking, and castrating. These will not predispose the animals to attack if they are performed at a time other than during the screw worm season and such causes are, therefore, subject to elimination.

Lambs.—What has been said of sheep with reference to the possibility of reducing or eliminating predisposing causes of myiasis such as needle grass, sore mouth, marking, birth, castrating, shear cuts, dehorning, and prickly pear, also applies to this class of animal.

In addition to those mention in the foregoing, docking is one of the more common predisposing causes of myiasis for this class. It is responsible for over 18 per cent of the cases recorded, but is subject to elimination since this operation can be done at a time when screw worm flies are not present.

Goats.—Over two-thirds of all predisposing causes of myiasis in goats are shear cuts. As stated in the discussion of sheep, the wholesale injuries in shearing are due to the type of clippers used by the shearers, the shearers' disregard of minor skin cuts in their haste to make the maximum wage, and the lack of an application of a good fly repellent to the wounds of the animal before it is liberated from the shearing pens. If these careless practices are discontinued, it seems certain that at least 75 per cent of the cases of myiasis caused by shear cuts will be eliminated.

Other predisposing causes that are subject to reduction or elimination are birth, castrating, and branding, since they can be timed or performed when screw worm flies are not active.

Lice as a predisposing cause can and should be totally eliminated. The eradication of lice by the well-known dipping method is practical and also of great economic importance on account of the severe and destructive injury to mohair by the lice.

Kids.—In this class, shear cuts, marking, birth, castrating, and lice are responsible for over 75 per cent of all myiasis. The means or methods for the reduction or elimination of these predisposing causes of myiasis pointed out in the discussion of sheep, lambs, and goats will apply also to this class.

Cattle.—The most common predisposing cause of myiasis in this class is injuries sustained from hooking while the animals are grazing in close quarters, crowded in pens, or crowded at feed or water troughs. Such injuries do not occur among dehorned animals. It seems clear, therefore, that the most common predisposing cause of myiasis in cattle is subject to elimination if all animals, excepting show stock, are dehorned while they are young and at a time of the year when blowflies are not present. Moreover, dehorning calves while they are very young is universally considered to be essential for the development of better beef animals.

Birth is responsible for over 10 per cent of all cases of myiasis in this class. Its occurrence at a time when screw worm flies are present predisposes both cow and calf. Furthermore, a beef calf born late or during the screw worm season is too young to be weaned the following winter and consequently the cow is compelled to nurse the calf when the range feed is usually insufficient to supply nourishment for her own normal requirements. Disease due to a greatly lowered resistance in a weakened and undernourished cow is often the result in these cases. The solution of this problem, together with the elimination of myiasis at birth, depends, therefore, on the selection of the proper time for the breeding period of the cows.

Other predisposing causes of myiasis subject to elimination are dehorning, branding, castrating, and marking. All these operations can and should be performed during the period when screw worm flies are absent.

Calves.—Nearly two-thirds of all cases of myiasis in this class are due to birth. Almost every calf dropped during the height of the screw worm season and a considerable percentage born at the beginning of the fly season become infested in the navel, and the animal, by licking the wound, transfers to the mouth worms which enter and destroy the gums. If the worms are not promptly removed, the teeth in the affected area are lost. Then dehorning, castrating, and, when necessary, branding and marking, must naturally follow soon after birth and at a time when they constitute excellent predisposing causes of myiasis. It is evident, therefore, that for the elimination of myiasis due to birth and certain operations which must follow, the selection of a proper time for the breeding period becomes of utmost importance for both the cow and the calf. Furthermore, from the livestock buyer's view, a so-called late calf is not ready for the feeder the following winter and is rejected or bought at a reduced price. It is true that even on the best-managed ranches a few cows are bred by a stray bull at a time which will result in a birth during the screw worm season. When this happens, myiasis after birth can be largely eliminated if dehorning, marking, and branding are delayed until the next worm-free period and if castration is performed with a Burdizzo type emasculator, which does not inflict a wound.

Horses and mules.—Wire cuts are the predisposing cause of over 61 per cent of all myiasis in this class. The careless scattering of barbed wire from fences which have been replaced during recent years by wolf-proof fencing for the protection of lambs and kids is apparently responsible for

the high incidence of this predisposition. This appears to apply only to the sheep and goat raising section of the State. The elimination of this careless practice, however, should not be overlooked in the consideration of methods aimed at the reduction of predisposing causes of myiasis for this class of animal.

The adoption of modified range practices as suggested under the other classes of animals listed above applies to this class for the reduction or elimination of myiasis due to birth and all man-made wounds.

SYSTEMATIC TRAPPING OF THE SCREW WORM FLIES³

The principal objective of this second investigation was to determine the effect of systematic trapping of the screw worm flies on the incidence of myiasis in domestic animals. During the three seasons when this study was made the test varied only in so far as unavoidable changes were concerned with reference to acreage and total animal population and concentration in the trapped and control areas. The number of acres, average number of all animals per month and per acre, number of traps, number of screw worm cases, and percentage of infestation among all animals in each of the two areas are given in the form of a condensed summary for each of the three years in question in table 3. Figure 2 shows the distribution of the traps in the test area. The 33,508 quarts of flies caught during one season (1932) are shown in figure 3. It has been estimated that 20 per cent of the flies are either destroyed in the traps by dermestid beetles or are

TABLE 3. Number of acres, animal population and concentration, number of traps, number of screw worm cases, percentage infestation of all animals, and percentage infestation ratio in the trapped and control areas for the period March 1 to October 31, 1931 to 1933, inclusive

| Year and area | Number of acres | Number of animals (average per month) | Number of animals (average per acre) | Number of traps in area | Number of cases in all animals | Percentage infestation of all animals | Percentage infestation ratio |
|---------------|-----------------|---------------------------------------|--------------------------------------|-------------------------|--------------------------------|---------------------------------------|------------------------------|
| 1931 | | | | | | | |
| Trapped | 154,879 | 104,789 | .68 | 661 | 1,654 | 1.58 | |
| Control | 144,860 | 82,261 | .57 | 0 | 2,401 | 2.92 | 1 to 1.85 |
| 1932 | | | | | | | |
| Trapped | 155,679 | 122,982 | .79 | 664 | 4,186 | 3.40 | |
| Control | 172,000 | 70,462 | .41 | 0 | 4,129 | 5.86 | 1 to 1.72 |
| 1933 | | | | | | | |
| Trapped | 152,479 | 124,292 | .82 | 656 | 1,695 | 1.36 | |
| Control | 113,580 | 70,686 | .62 | 0 | 2,399 | 3.39 | 1 to 2.49 |

³ Since the studies reported in this paper were completed Cushing and Patton (5) discovered that there are two species of screw worms which previously had been confused under the name *Cochliomyia macellaria*. They named the new species *americana* and suggested that it probably feeds and oviposits on living tissue only and is not attracted to meat baits. During the season of 1934, however, the writer caught both species in meat-baited traps in the same area where the above tests were made, the proportion being one *americana* to 2,427 *macellaria*. This ratio may or may not indicate their relative abundance in nature in the area tested. During the same season *macellaria* was found infesting wounds in domestic animals. The true economic status of this species has not been determined.

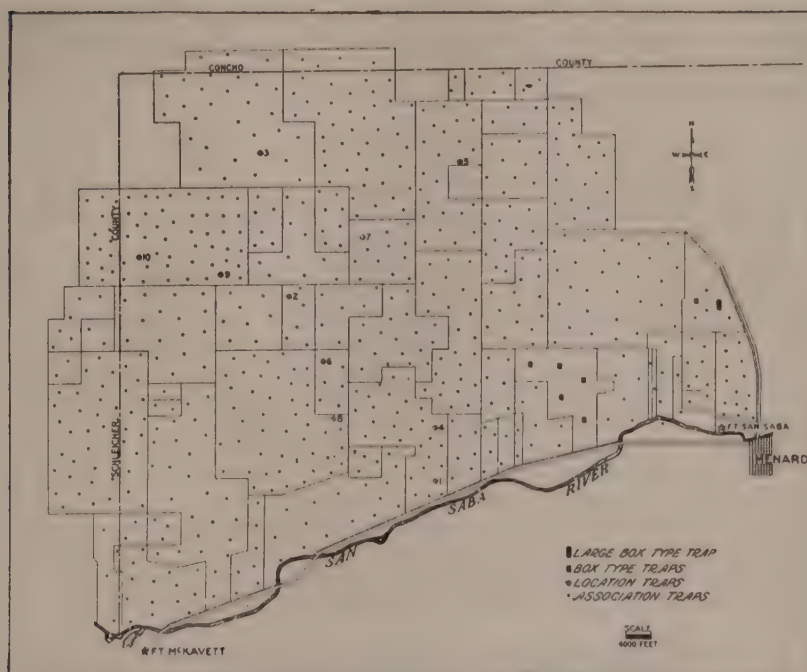


Fig. 2. Distribution of the flytraps in the test area.

killed by the larvicide in the bait before they enter the traps and are therefore not measured. If this 20 per cent were added to the measured catch, the total would exceed 40,000 quarts of flies destroyed in the trapped area during that season.

In the test in 1931 to determine the effect of systematic trapping of screw worm flies on the incidence of myiasis in domestic animals, the percentage of screw worm infestations in the trapped area was 1.58 among 104,789 animals as compared with 2.92 per cent among 82,261 animals of all classes in the control area. In 1932 these percentages were 3.4 and 5.86, respectively, and in 1933, 1.36 and 3.39, respectively. It is obvious that slight variations must exist in two areas as large as these in question, but it is not likely that they would constitute more than a small relative variation in the aggregate. There appears to be no factor other than the operation of the flytraps to account for the reduction in the percentage of infestation in the trapped area. It should be noted that although the percentage of infestation in 1932 was slightly more than double that of 1931 (table 3), the proportion of the reduction of myiasis in the two areas remained virtually the same. This seems to indicate that trapping is equally effective during years of mild or of moderately heavy incidence of myiasis.

The results obtained in this test indicate that large-scale, systematic trapping has considerable merit as a supplemental method for controlling screw worm flies. However, the expense of the operation as carried out

in this experiment is too great to justify its recommendation for general adoption. Fly trapping may have a distinct practical value if a more attractive bait for *Cochliomyia americana* is found and if a cheaper method of operation is developed.

SUMMARY

Losses estimated at \$5,000,000 annually have been caused by the screw worm fly, *Cochliomyia americana*, in the Southwest, the attack by the flies being made possible by wounds or sores.

The author, in making a five-year study of the predisposing causes of attack on more than 75 ranches in Menard County, Texas, found that among sheep and lambs wounds caused by needle grass gave occasion for attack by the flies more than any other one cause; among goats and kids the predominating cause was shear cuts; among cattle it was injuries by the horns of other cattle. Calves suffered most as a result of exposed tissue at birth, and horses and mules from wire cuts.

Several of the more common causes of attack are due to ranch practices that can be changed or better timed. Particularly stressed are greater care in shearing, dehorning of cattle while they are young, removing and disposing of old barbed wire from dismantled fences instead of leaving it on the ground, and the timing of dehorning, castrating, and branding of animals, and of breeding, so that as little as possible of open sores or wounds will be exposed to the flies during the season of their abundance.

The percentage of infestation was found to range from 1.38 in 1930 to 4.30 in 1932. The possibility of reducing this infestation by trapping the flies was tried out over an area of approximately 155,000 acres of ranch land in Menard County, Texas. The apparent reduction in percentage of infestation, as compared with that on a similar area of about equal size in the same vicinity, was from 2.92 to 1.58 in 1931, from 5.86 to 3.40 in 1932, and from 3.39 to 1.36 in 1933.

Trapping seemed equally effective in years of mild or moderately heavy infestation. The expense of trapping as carried out in these tests, however, is too great to justify a recommendation for its general use. If a more attractive bait for *Cochliomyia americana* is found and if a cheaper method of operation is developed, fly trapping may become of distinct practical value as an aid to the control of this serious pest of livestock.

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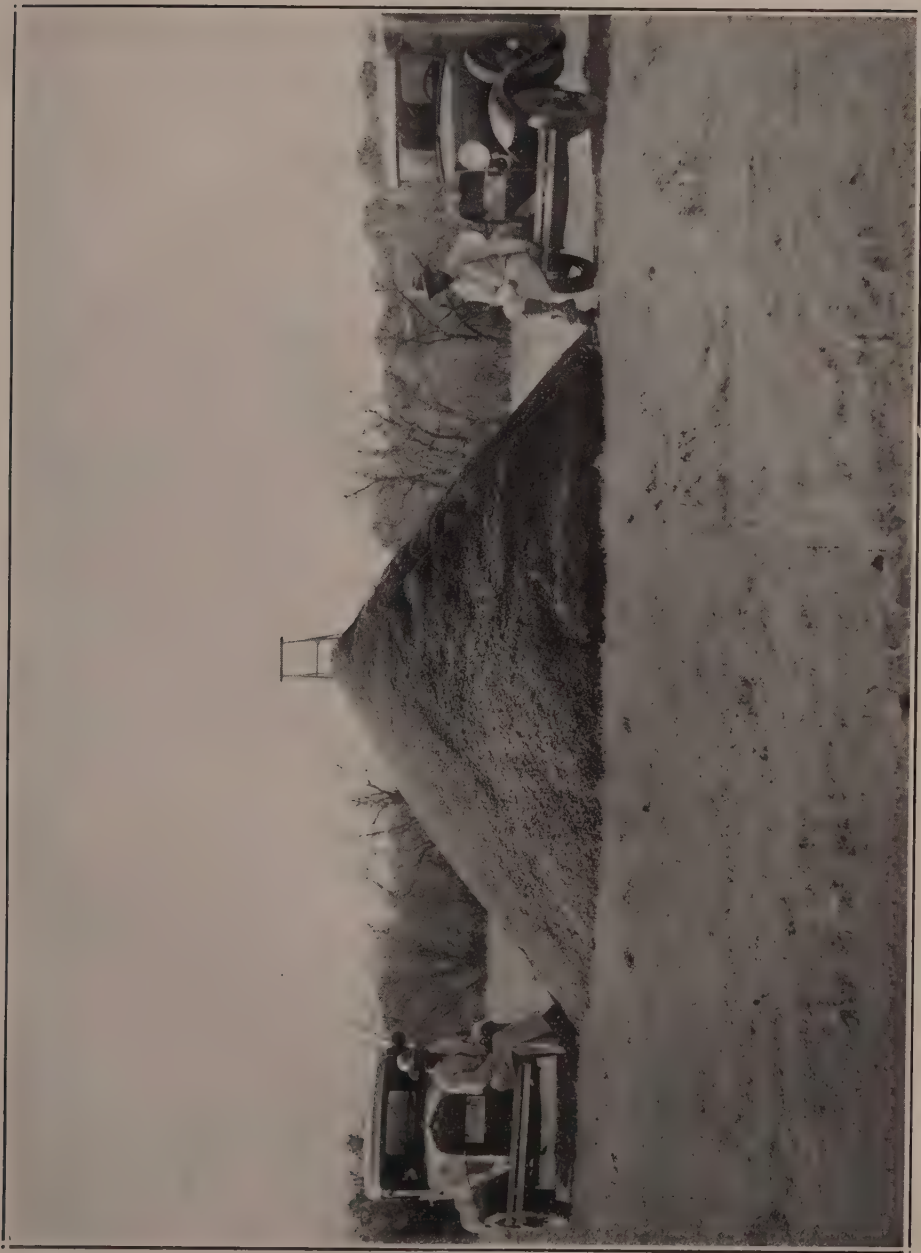
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PLATE I

Thirty-three thousand five hundred eight quarts of flies caught in 664 traps during the period March 1 to October 31, 1932, on 155,679 acres of ranch land in Menard County, Texas. The two trappers shown in the picture, Mr. S. M. Perry (left) and Mr. D. C. Thurman, took care of all the traps.

PLATE I



SEASONAL APPEARANCE AND RELATIVE ABUNDANCE OF FLIES ATTRACTED TO BAITED TRAPS

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Systematic fly trapping in the vicinity of Ames, Iowa, was carried on during 1933 and 1934 to determine the date of appearance and the relative and seasonal abundance of the various species of flies attracted to traps baited with liver. Roberts (1) operated fly traps in the vicinity of Ames, Iowa, from October 1, 1927, to May 5, 1928, but no published records of fly trapping studies in the Middle West during the summer months have been noted.

Four standard, cone type, screen fly traps (2) were placed approximately 150 yards apart, on the laboratory premises in the following locations: (1) Attached to the side of the barn; (2) on a fence post in an unshaded place near a small stream; (3) five feet above ground on the trunk of a large walnut tree close to the city dump and bordering a woodland; and (4) on a fence post near the center of a 20-acre pasture.

Each trap was baited with 2 pounds of pork liver, except in relatively few cases where pork liver was not available. In such cases beef liver was used. Enough water was added to fill the bait pan. In 1933 the traps were emptied every 7 days and the bait was renewed every 14 days. In 1934 the traps were emptied and the bait renewed every 10 days. The flies from each trap were measured volumetrically, and 500 flies were taken as a representative sample from the catch of each trap for the determining of genera or species. No sex determinations were recorded. The combined results from the four traps are used in the data for each season.

SEASONAL APPEARANCE

Two traps were started with the first appearance of flies on March 3, 1933, and frequent observations were made thereafter to determine the date of appearance of the various species. Less frequent observations were made in 1934, but no appreciable differences in the dates of appearance were noted.

Table 1 gives a list of the flies trapped in 1933 and the dates of their appearance.

SEASONAL ABUNDANCE

The total catch of flies at regular 7-day intervals in 1933 is shown in figure 1, and the total catch at regular 10-day intervals in 1934 is shown in figure 2. The highest catch in 1933 from the four traps, 137 pints, was recorded for the period July 25-31; the next highest catch, 136 pints, was for the period July 11-17. The total catch for the 1933 season was 1,867 pints.

In 1934 the highest catch, 210 pints, was recorded for the 10-day period ending July 21, and the second highest catch, 198 pints, for the 10-day

period ending August 10. The total catch for the 1934 season was 1,785 pints.

TABLE 1. List of flies trapped in 1933 and dates of their appearance

| | |
|---|----------------|
| <i>Phormia regina</i> Meig. | March 3 |
| <i>Calliphora erythrocephala</i> Meig. | March 11 |
| <i>Pollenia rudis</i> Fab. | March 12 |
| <i>Scatophaga</i> spp. | " |
| <i>Fannia</i> spp. | " |
| <i>Muscina assimilis</i> Fall. | March 13 |
| <i>Cryptolucilia</i> sp. | " |
| <i>Cynomyia cadaverina</i> Desv. | March 20 |
| <i>Lucilia sericata</i> Meig. | March 31 |
| <i>Muscina stabulans</i> Fall. | " |
| <i>Musca domestica</i> L. | April 1-10 |
| <i>Calliphora coloradensis</i> Hough. | " |
| <i>Hydrotaea dentipes</i> Fab | " |
| <i>Lucilia illustris</i> Meig. | April 24-May 1 |
| <i>Sarcophaga</i> female (undetermined) | " |
| <i>Sarcophaga lherminieri</i> R. D. | " |
| <i>Chrysomya demandata</i> Fab. | " |
| <i>Lucilia sylvarum</i> Meig. | May 8-15 |
| <i>Ophyra leucostoma</i> Wied. | " |
| <i>Sarcophaga haemorrhoidalis</i> Fall. | May 15-22 |
| <i>Protophormia terraenovae</i> Desv. | " |
| <i>Cochliomyia macellaria</i> Fab. | " |
| <i>Sarcophaga ventricosa</i> v. d. W. | " |
| <i>Sarcophaga bullata</i> Park. | " |
| <i>Sarcophaga pusiola</i> v. d. W. | " |
| <i>Sarcophaga utilis</i> Ald. | May 22-29 |
| <i>Graphomyia maculata</i> Scop. | " |

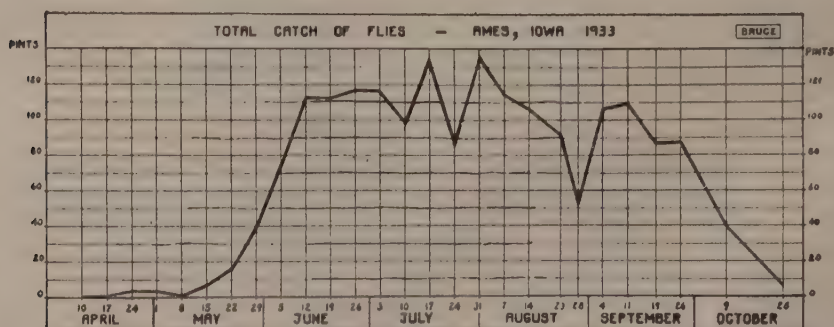


Fig. 1. Total volume (pints) of flies caught in four cone type traps, Ames, Iowa, 1933.

RELATIVE SEASONAL ABUNDANCE

The most abundant species of flies in 1933 was *Musca domestica*, *Phormia regina*, *Lucilia* spp., *Cochliomyia macellaria*, and *Cynomyia cadaverina*. The relative seasonal abundance of these species is shown in figure 3.

The relative seasonal abundance of the most abundant species for 1934, *Musca domestica*, *Phormia regina*, *Cochliomyia macellaria*, *Lucilia* spp., and *Cynomyia cadaverina*, is shown in figure 4.

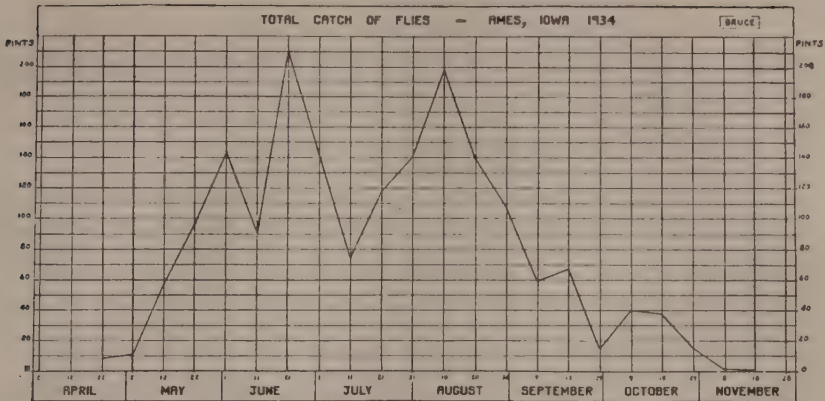


Fig. 2. Total volume (pints) of flies caught in four cone type traps, Ames, Iowa, 1934.

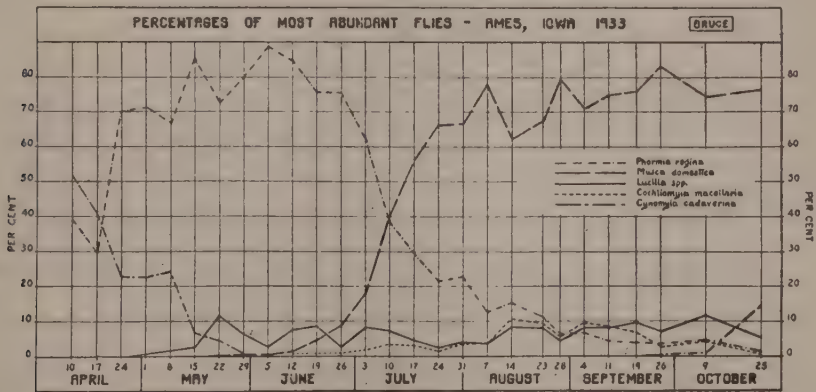


Fig. 3. Relative abundance, seasonally, of the more abundant species of flies caught in traps, Ames, Iowa, 1933.

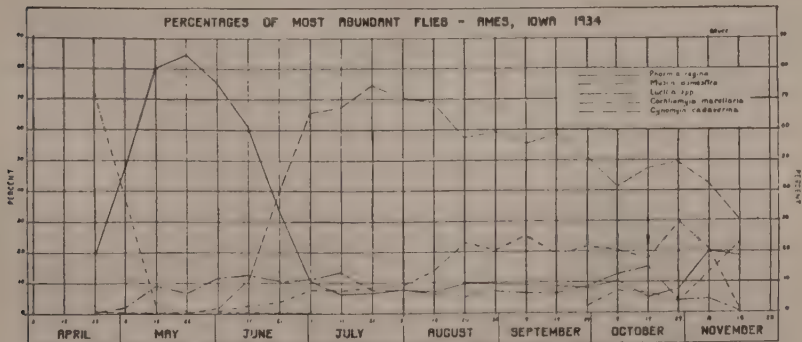


Fig. 4. Relative abundance, seasonally, of the more abundant species of flies caught in traps, Ames, Iowa, 1934.

COMPARATIVE ABUNDANCE

Table 2 gives a list of the flies trapped and their relative abundance for 1933 and 1934.

TABLE 2. Flies trapped and their relative abundance in 1933-1934

| Species | 1933 Percentage | 1934 Percentage |
|---------------------------------|--------------------|--------------------|
| <i>Musca domestica</i> | 42.28 | 40.84 |
| <i>Phormia regina</i> | 39.56 | 25.03 |
| <i>Lucilia</i> spp. | 6.18 | 7.90 |
| <i>Cochliomyia macellaria</i> | 3.29 | 10.88 |
| <i>Cynomyia cadaverina</i> | 2.82 | 7.58 |
| <i>Ophyra leucostoma</i> | 2.52 | 1.29 |
| <i>Sarcophaga</i> spp. | 1.24 | 2.54 |
| <i>Mucina</i> spp. | 0.68 | 0.08 |
| <i>Hydrotaea dentipes</i> | 0.52 | 0.30 |
| <i>Fannia</i> spp. | 0.52 | 0.74 |
| <i>Scatophaga</i> spp. | 0.30 | |
| <i>Calliphora</i> spp. | 0.24 | 1.65 |
| <i>Morellia micans</i> | 0.08 | 0.00 |
| <i>Mydea</i> sp. | 0.00 | 0.06 |
| <i>Pollenia rudis</i> | 0.05 | 0.04 |
| <i>Protophormia terraenovae</i> | 0.03 | 0.02 |
| <i>Cryptolucilia</i> spp. | 0.02 | 0.10 |
| <i>Chrysomya demandata</i> | 0.02 | 0.12 |
| <i>Euxesta</i> sp. | 0.00 | 0.01 |
| <i>Graphomyia maculata</i> | 0.01 | 0.00 |
| <i>Synthesiomyia</i> sp. | 0.00 | 0.01 |
| Undetermined | 0.48 | 0.04 |

Four species of *Lucilia* were collected in 1934. These, in order of their abundance, were: *Lucilia sericata* L. *illustris*, L. *sylvorum*, and L. (*australis* Towns.) *caeruleiviridis* Macq. Four species of *Calliphora* were collected in 1934, namely, *Calliphora erythrocephala*, *C. coloradensis*, *C. viridescens* Desv., and *C. vomitoria* L. Of these, *Calliphora erythrocephala* was most numerous and *C. coloradensis* and *C. viridescens* were present in about equal numbers. Only one specimen of *C. vomitoria* was taken.

Twenty species of the genus *Sarcophaga* were collected during the two seasons, as follows: *Sarcophaga bullata* Park., *S. stimulans* Walk., *S. importuna* Walk., *S. pusiola* v. d. W., *S. haemorrhoidalis* Fall., *S. misera* var. *sarracenioides* Ald., *S. pectinati* Ald., *S. barbata* Thom., *S. planifrons* Ald., *S. utilis* Ald., *S. sinuata* Meig., *S. kellyi* Ald., *S. rapax* Walk., *S. ventricosa* v. d. W., *S. lherminieri* R. D., *S. latisetosa* Park., *S. cimbicis* Towns., *S. sulcata* Ald., *S. galeata* Ald., and *S. impar* Ald.

DISCUSSION

The first flies trapped during the 1933 season were taken on March 3 (table 1). *Phormia regina* was the first to appear. Other early-appearing species were *Calliphora erythrocephala*, *Pollenia rudis*, *Scatophaga* spp., *Fannia* spp., and *Cynomyia cadaverina*. *Musca domestica*, *Lucilia* spp., *Sarcophaga* spp., *Cochliomyia macellaria*, and other species made their appearance somewhat later.

Figures 3 and 4 show the comparative seasonal abundance of the more abundant flies. *Musca domestica*, *Phormia regina*, *Lucilia* spp., *Cochliomyia macellaria*, and *Cynomyia cadaverina*. It is interesting to note that *Phormia regina* is abundant during the early part of the season and gradually decreases during the remainder of the season. *Musca domestica*, on the other hand, is present in comparatively small numbers during the early part of the season, but shows a decided increase during the latter part of the season. The increase in numbers of *M. domestica* occurs at about the time that *Phormia regina* shows a decrease. *Cynomyia cadaverina* is present in comparatively large numbers during the late winter and early spring, but practically disappears during the warmer months, and again shows a decided increase during the fall. The comparative abundance of *Lucilia* spp. does not show the fluctuation that is found in *P. regina*, *M. domestica*, and *C. cadaverina*. *Cochliomyia macellaria* is present in comparatively small numbers during the early part of the fly season and shows a gradual increase during the last half of the season.

The comparative abundance of the various species of flies for 1933 and 1934 is shown in table 2. The most abundant species, *M. domestica*, *P. regina*, *Lucilia* spp., *Cochliomyia macellaria*, and *Cynomyia cadaverina*, are the same for the two seasons. *Musca domestica* was the most numerous both seasons and the percentage of the total shows no significant difference for the two seasons. *Phormia regina* ranked second both seasons, but the percentage of the total catch was much less in 1934. In 1933 the percentage of the total was 39.56 and in 1934 it was 25.03. *Lucilia* spp. ranked third in 1933, with a percentage of 6.18, and fourth in 1934, with a percentage of 7.90. *Cochliomyia macellaria* showed a decided increase in 1934 over 1933. For 1933 the percentage of the total was 3.29 and in 1934 it rose to 10.88. *Cynomyia cadaverina* ranked fifth in order of abundance in the two seasons; in 1933, however, the percentage of the total catch was only 2.82 as compared with 7.58 for 1934. The five most abundant flies represented 94.13 per cent of the total in 1933 and 92.22 per cent of the total in 1934.

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SUMMER STUDIES ON THE COTTONTAIL RABBIT (*SYLVILAGUS FLORIDANUS MEARNSI* (ALLEN))¹

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As a game animal and as food for several carnivores the cottontail rabbit in several species and many subspecies has recently come into greater prominence. The cottontail of central Iowa, and probably of all of the state, is *Sylvilagus floridanus mearnsi* (Allen), according to Nelson (1909) and Stoner (1918). A deceased individual found on the road near the area of study reported in this paper meets the description of this subspecies. In several counties of Iowa, hunters have not been able to find cottontails in satisfactory numbers in the past three winters, and consequently, among others the author has been called to devise concrete practices that may bring about an increased population of this rabbit.

Inquiry into the causes of the decline in the rabbit population has not yielded much evidence. Mr. Myrle L. Jones of Pomeroy, Iowa, reports that of 54 cottontails taken in northwest counties during the winter of 1933-1934, 35 individuals had the wart disease. Mr. T. G. Scott, Extension Specialist in Wildlife Management, Iowa State College, learned that in several counties of the southwest section of the state many rabbits taken by hunters during the early part of the winter of 1935-1936 exhibited warts. As to the extent of tularemia in rabbits or the effects of external and internal parasites on rabbits no definite information is at hand. But Mr. Jones also reports that in the winter of 1933-1934 several days of hunting in southeast counties yielded less rabbits than were taken in the same amount of time in similar weather in the northwest section, and of the 20 individuals taken only one rabbit possessed warts. To gather more concrete data in regard to the causes of the low numbers of cottontails as reported by many hunters and hence assumed to have some basis of truth, arrangements are being made to have slow-moving, and hence presumably diseased, cottontails taken by Conservation Officers and sent to Iowa State College for diagnosis of tularemia, other diseases and parasites.

Two of the first problems confronting the investigator were to ascertain the numbers of individual rabbits on a given area of land and to learn the extent of use of this area by the resident rabbits. Connected with these problems questions concerning the cruising radius of the animal, and the nature, quality and distribution of cover arose. And in the course of the study certain timely observations in behavior of the cottontail were made somewhat incidentally.

A perusal of literature revealed much interesting general information about cottontails. Although for the game manager the facts are not stated in sufficiently exact terms as yet to enable him to devise practices that will maintain a desired number of rabbits on a given area, this general

¹ Supported in part by a grant from the Industrial Science Research Fund of Iowa State College for the study of Management of Cottontail Rabbits; cooperating with the Iowa Conservation Commission and the United States Bureau of Biological Survey.

information furnishes many clues for points of attack upon the problems of cottontail study. Seton (1929) in his detailed summary of the literature on cottontails and supplemented by personal observations states that he thinks "it is probable that a cottontail, unless driven afar by hounds or foxes, spends its whole life within the limits of an acre."

The first concrete suggestion as to a method of attack upon the problem of ascertaining the numbers of rabbits on a given area came from Vorhies and Taylor (1933). They state that rabbits regularly defecate while they are feeding or very soon after, and that field observations indicate similar habits on the part of hares in the wild. Observations on cottontails lead one to say the same for them. These investigators also found a significant relationship between numbers of fecal pellets and abundance of jack rabbits on a given area, but were not able to determine the absolute numbers of individuals on a given range by the pellet-count method. The method consists of counting the pellets on one-square-foot-areas at fifty-foot intervals over entire large plots of rabbit inhabited land. At each interval the square foot is determined by placing a circular hoop on the ground.

For purposes of this study the author chose a 15.5-acres preserve including the college apiary farm and a portion of the college seedling orchard. As pellet-counts at 50-foot intervals, when plotted, did not show a distribution that would furnish clues as to the numbers of resident cottontails, counts were repeated at 25-foot intervals, July, 1935. In each count the area involved was that within the bounds of a circular wire hoop enclosing one square foot, and none of the pellets were removed from any of the areas of the counts.

In table 1 the plant cover and acreage of the several tracts of the preserve are shown together with total numbers of square-foot-counts and numbers of pellets.

TABLE 1. *Data from several tracts studied*

| Num- ber of tract | Plant cover | Acreage | No. of sq. ft. counts | Total nu- ber of pellets |
|-------------------------|---|---------|-----------------------------|--------------------------------|
| 1 | Apple trees, bluegrass, red clover, weeds | 4.9 | 345 | 313 |
| 2 | Tall alfalfa | 3.2 | 230 | 63 |
| 3 | Weedy bluegrass (pastured) | 5.1 | 359 | 15 |
| 4 | Tall bluegrass | 1.6 | 114 | 37 |
| 5 | Bluegrass of mowed lawns | .7 | 54 | 0 |

In tract No. 1, the apple trees vary in height from 6 to 15 feet and stand about 2 rods apart. Over one-fourth of the tract the branches from neighboring trees meet; elsewhere the branches are up to 25 feet apart. Three-fourths of the ground cover was bluegrass (*Poa pratensis*) of a thin stand about 6-8 inches tall and nearly one-fourth was weedy red clover about 8 inches tall and of a thick stand. A gully 2-3 feet deep and filled with brush runs diagonally half way into the orchard. In an area of .10

acre at one corner of the tract furrows had been plowed 5 feet apart to check soil-washing at the end of the gully. There tall weeds, chiefly wild lettuce (*Lactuca scariola*), grew up and with tall bluegrass between the furrows cover attractive to rabbits was formed. In tract No. 2, the alfalfa was about 20 inches tall. No pellets were found in those parts of the tract where the stand was 16 stalks or more per square foot. In such parts the stems were tangled and somewhat moldy. About one-half of the tract was in such condition. In tract No. 3, the most rank weedy cover, chiefly hedge mustard (*Sisymbrium officinale*) and lamb's quarters (*Chenopodium album*) was about 2 feet tall. The bluegrass over about three-fourths of the tract was closely grazed. In tract No. 4, the bluegrass was of a quite clean stand with 55 stalks per square foot and 18 inches tall, but furnishing little protective cover or shade. The pellets of this tract were smaller than most of the pellets of the other tracts and 3 young rabbits, about one-fourth grown, were seen daily close to this bluegrass. They spent much time under several large boxes in the lawn at the edge of the bluegrass and were not as yet large enough to wander out far into individual territories such as are discussed later for adult cottontails. In tract No. 5 are included several small pieces of lawn connected by paths and roadways, which were used by the rabbits early mornings and evenings for exercise, play, mating activities, and movement to and from shelter. A part of the seedling orchard of about three acres in extent, cultivated to be free of ground cover and not included in the 15.5 acres of these studies, was used a great deal for like purposes.

To what extent open paths and roadways are needed by rabbits the author is not able from these studies to state. No evidence of cutting definite paths as one sees often in tangles of briars and willows was seen anywhere on the preserve. It is assumed that the several roadways and pieces of lawn which cut the preserve into the several tracts sufficed for considerable freedom in movement as well as for play.

By walking through the several tracts at intervals of one rod, 6 to 10 times, both with and without a trained dog (of beagle-terrier cross), by looking and poking underneath shrubbery and other cover as many times, and by observation early mornings and evenings when weather permitted, the number of rabbits was ascertained as reported to have been seen in table 2; only those regularly seen are reported in the table. One rabbit was jumped from a form at the edge of a thick stand of alfalfa, but no pellets were found nearby and, as it could not be accounted for again by sight, it is not reported in table 2 for tract No. 2. One small young rabbit was seen in tract No. 2 within the territory of an adult. A few times two rabbits were observed in tract No. 3. More often a rabbit from tract No. 3 met a second rabbit in the cultivated orchard just off the tract. Only one rabbit nest was seen, unoccupied, near an adult's territory of tract No. 3. A dead rabbit, about one-fourth grown, was found in tract No. 1.

Observations on the rabbits, as stated in the preceding paragraph, showed them to occupy mostly during the daytime limited individual territories which were disclosed also by groupings of pellet-areas as shown in pellet counts. The numbers of these territories for each tract are shown in table 2. A territory is considered as bounded by the periphery connecting the outermost one-square-foot-areas that showed pellets, which in turn inclosed a number of other areas which showed pellets. Spaces with

TABLE 2. *Cottontail territories*

| Number of tract | Number of daytime territories | Average territorial acreage | No. of adult rabbits seen several times | Number of forms observed |
|-----------------|-------------------------------|-----------------------------|---|--------------------------|
| 1 | 4 | .58 | 4 | 2 |
| 2 | 4 | .11 | 4 | 4 |
| 3 | 1 | .23 | 1 | 1 |

pellet-free areas which intervened between such territories aided in setting territorial boundaries. The number of rabbits frequently seen in such positions and at such times as to make a sight census of 9 adults reasonably accurate corresponds with the number of territories, 9, as shown with pellet distribution. During the warmer mid-day hours when the temperature was over 90° F., the cottontails were not easily put to flight, as a number of times the animals were seen to merely move a few feet to one side of the direct path of the observer and even the dog. And when noticed by the dog, which had some difficulty in running rapidly through dense cover, the rabbit in leisurely fashion made its way to safer shelter such as a brush pile. The rabbits were seldom found at shrubbery, brush piles, or such safety centers during the warmer daylight hours.

Not all of the forms were discovered. In three cases a rabbit was jumped from the same form several days in succession. One of the rabbits after a week left the form and it is thought that some individual made a form within a few feet of a brush pile. Each form consisted of two parts. One part large enough for a resting rabbit was entirely bare of all plant growth and rubbish, while in the second half, as large as the first, the ground was covered with a thin layer of leaves and portions of stems.

In an attempt to discover factors other than food cover which might cause the rabbits to choose and occupy a given territory the distances between the most distant point of the territorial peripheries and the neighboring protective cover that was sought when they were pursued by the dog were measured. Such cover included shrubbery, brush piles, drain tile, culverts, and entrances under small buildings. These average distances are shown in table 3.

TABLE 3. *Distances to protective cover*

| Number of tract | Average number of rods from shrubbery to farthest point of territory | Average number of rods from brush pile, etc., to farthest point of territory |
|-----------------|--|--|
| 1 | 17.9 | 16.7 |
| 2 | 5.7 | 12.4 |
| 3 | 13.6 | 10.6 |

The average number of rods from shrubbery to the farthest point of territorial periphery was obtained in this manner. From the shrubbery nearest the territory of each rabbit in a tract the distance to the farthest point of the pellet-count periphery of its territory was measured. It is as-

sumed that this distance indicated the farthest distance a rabbit would be required to traverse in seeking safety from a pursuing enemy such as a dog, if the pursued animal were to use the shrubbery to elude the pursuer. Then these distances for all the rabbits of a tract were added and the sum was divided by the number of rabbits to obtain the average distance of the farthest point of territory from shrubbery. In like manner the average distance from the farthest point of territory to brush pile, culvert, or similar object which afforded a greater degree of safety than shrubbery was obtained.

Although alfalfa and red clover evidently are choice food plants for the cottontail, they are not insurance against damage to garden crops at all times. In early spring the rows of peas and lettuce of a small garden neighboring the alfalfa tract showed 5 to 6 feet of damaged plants. After the alfalfa had grown to a height of 10 inches and the remaining garden plants were several inches tall, rabbit damage ceased in the garden.

In summary, 9 adult and 4 young rabbits were found on 15.5 acres in June and July, 1935, a density of one rabbit to 1.1 + acres. It is suggested that the pellet-count method may be used not only to determine the relative numbers of cottontails on a given area in summer, but to learn the approximate area of feeding cover that each individual uses and its requirements as to safety cover. This method will aid in cover evaluation, and the findings of these studies as to such requirements conform in several ways with the suggestions of Trippensee (1934). They differ in that alfalfa, at least, with over 64 stalks per 4 square feet would score 0 rather than 10 in density. The pellet-count method aids in determining the relative value of several types of cover in relation to each other, and may make possible more concrete suggestions as to placements of safety or escape cover in relation to food cover, thereby supplementing Trippensee (1934).

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THE RING-NECKED PHEASANT AS A NESTING PARASITE OF OTHER GAME BIRDS¹

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For many years ornithologists and naturalists have known of the parasitic nesting habits of many birds, including certain ducks. Undoubtedly on the original prairies of Iowa some ducks parasitized nests of other species. It is unknown to what extent the Prairie Chicken, the dominant upland game bird of the early pioneer days, showed parasitic nesting tendencies toward waterfowl and other birds that nested on the prairies. Probably this grouse was not troublesome in this way to other birds because it appears that the grouse was quite selective in choosing its nesting environment.

With the extensive development of intensive agriculture in Iowa the native prairies have almost entirely disappeared before the plow. The exclusion of the prairie flora that supplied nesting cover for the Prairie Chicken spelled its doom. The decline of this bird, probably having begun between 1875 and 1890, continued to the point where at present it is probably no longer a breeding bird in Iowa. Several trained ornithologists have spent many hours in the field in the past several years in regions where this grouse was said by local citizens to be nesting. But no Prairie Chicken nests have been observed in Iowa for several years by reputable observers. There has not been an open hunting season on this bird in Iowa since 1916. It is interesting to note that the last open season took place at approximately the same time that Iowa's extensive drainage program was completed.

For a number of years northern Iowa was without an upland game bird in plentiful numbers. There was not a gallinaceous game bird that could thrive in the great corn producing area that arose from the prairies. The Prairie Chicken would not be confined to nesting areas found only along fence rows and around the few remaining sloughs and potholes that somehow escaped drainage. A change in the situation began in 1900 when a windstorm destroyed a pheasantry, releasing about one thousand Ring-necked Pheasants (Leopold, 1931). Following many other plantings this newly introduced upland game bird has thrived very well as a result. Iowa has been able to have an annual open season on this bird since 1927, with the exception of a closed season in 1928. Although the pheasant has been acclimating itself to Iowa during a period of thirty-five years there has been but little research to ascertain the effects of the bird upon our native plants and animals. Will any beneficial effects to our flora and fauna be produced by the Ring-necked Pheasant? Will any detrimental effects follow as the bird establishes itself in a niche in our native fauna?

¹ Journal Paper No. J312 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 496.

² Iowa Agricultural Experiment Station and the Iowa Conservation Commission, cooperating, with the United States Bureau of Biological Survey.

Data were obtained in the course of waterfowl studies in northwest Iowa (Errington and Bennett, 1934) during the past four years relative to the relationship between nesting pheasants and nesting water birds. In many cases pheasants utilize Puddle Duck nesting areas as readily as do ducks. (In Iowa the cost of restoring waterfowl nesting areas may be lowered because of the pheasant production that takes place on such restored areas. In 1934 the author observed one pheasant nest per acre on a one-hundred-acre tract of a duck nesting area in Dickinson County, Iowa. On this basis, if such a tract was being purchased for a game bird nesting area, certainly not more than half of the expense could rightfully be charged up to waterfowl.) During the entirety of the duck studies, 340 Puddle Duck nests were observed in Iowa. Of this number 16 contained pheasant eggs. (See table 1.)

TABLE 1. *Parasitized nests*

| Number and bird | | No. eggs of nest building species | Pheasant eggs | Eggs of nest building species hatched | Pheasant eggs hatched |
|-----------------|--------------------|-----------------------------------|---------------|---------------------------------------|-----------------------|
| 1. | Mallard | 6 | 3 | 6 | 0 |
| 2. | " | 7 | 3 | 7 | 0 |
| 3. | " | 7 | 3 | 7 | 0 |
| 1. | Blue-winged Teal | 11 | 1 | 11 | 0 |
| 2. | " " " | 11 | 4 | 0 | 0 |
| 3. | " " " | 6 | 3 | 6 | 1 |
| 4. | " " " | 4 | 3 | 0 | 0 |
| 5. | " " " | 9 | 1 | 0 | 0 |
| 6. | " " " | 4 | 6 | 0 | 0 |
| 7. | " " " | 14 | 11 | 0 | 0 |
| 8. | " " " | 4 | 3 | 0 | 0 |
| 9. | " " " | 8 | 1 | 8 | 1 |
| 10. | " " " | 5 | 5 | 0 | 0 |
| 11. | " " " | 3 | 3 | 3 | 0 |
| 12. | " " " | 7 | 1 | 7 | 0 |
| 1. | Shoveller | 6 | 3 | 6 | 0 |
| 1. | Virginia Rail | 6 | 1 | 6 | 0 |
| 1. | King Rail | 5 | 3 | 0 | 0 |
| 1. | European Partridge | 12 | 3 | 12 | 3 |
| 2. | " " | 9 | 4 | 0 | 0 |
| 3. | " " | 11 | 2 | 11 | 0 |
| 4. | " " | 7 | 6 | 0 | 0 |

The largest clutch found was that of a Blue-winged Teal containing fourteen teal eggs and eleven pheasant eggs. In only one case did the pheasant eggs outnumber the duck eggs. A number of single pheasant eggs were found from several inches to several feet from duck nests. As Blue-winged Teal outnumbered all other nesting Puddle Ducks combined, more pheasant eggs were deposited in teal nests than in the nests of other ducks. Whether the duck nests were used as dumping nests, merely through carelessness, or with nesting intentions by the pheasant,

it would be difficult to ascertain. In all cases the nests were occupied by ducks and not by pheasants. In two instances the pheasant eggs hatched at the same time as those of the duck. One can imagine the situation that must have arisen when the duck took her brood to the marsh! With the exception of the teal nest containing fourteen teal eggs and eleven pheasant eggs, all of the duck clutches contained less than the average number of duck eggs found in nests not parasitized by pheasants. It appeared that laying on the part of the duck ceased when the nest became filled, and, after incubation began, the depositing of pheasant eggs in the duck nest ceased. For a total of 227 normal Blue-winged Teal nests, the average clutch contained 9.44 eggs; for 84 Mallard nests the average was 10.6 eggs per clutch; for 13 Shoveller nests the average was 9 eggs per nest. On this basis the teal nests containing pheasant eggs averaged 7.16 duck eggs per clutch; the Mallard nests averaged 6.66 duck eggs; and the Shoveller nest contained 6 duck eggs. From these data, although few, it appears that pheasant eggs deposited in a duck nest reduce the potential number of duck eggs. The observations thus far have not revealed any physical strife between nesting pheasants and nesting ducks. Within a few feet of many duck nests, pheasant nests have very frequently been observed. If there were any territorial difficulties between nesting ducks and pheasants, the effects were not in evidence.

While in the pursuit of duck nesting data the author came across a number of Virginia Rail and King Rail nests. One Virginia Rail nest with six rail eggs and one pheasant egg was observed. (See table 1.) This nest was built of sedge, *Carex* sp., in a very damp habitat. One King Rail nest with five rail eggs and three pheasant eggs was found. The nest, built of blue flag, *Iris versicolor* L., was situated in a slough with two inches of water at the particular spot where the nest was located. The pheasant eggs did not hatch in either of the rails' nests.

Between forty and fifty European Partridge nests were observed. Four of these nests contained pheasant eggs. (See table 1.) In one nest containing twelve partridge eggs and three pheasant eggs, all the eggs hatched. The pheasant eggs in the other nests did not hatch.

SUMMARY

1. Approximately 4.7 per cent of the Puddle Duck nests in the prairie area of Iowa are parasitized by the Ring-necked Pheasant.
2. Apparently pheasant eggs deposited in duck nests reduce the potential number of duck eggs.
3. A small percentage of pheasant eggs deposited in duck nests hatch.
4. There is no apparent strife between nesting ducks and nesting pheasants.
5. A small percentage of King Rail nests, Virginia Rail nests, and European Partridge nests are parasitized by pheasants.

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A NEW GENUS AND SPECIES OF PODOPIDAE AND A NEW COENUS (HEMIPTERA: SCUTELLEROIDEAE)

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While arranging some miscellaneous hemiptera, now almost five years ago, the writers set aside the forms described below with the notation that apparently they were new to America and that efforts should be made to secure more of each. During the interim collecting trips have been made into Mississippi, Arkansas, Louisiana and Texas, correspondence has been carried on with other hemipterists, visits have been made to the Carnegie Museum and the National Museum and collections from the above named and adjoining states have been examined with great interest whenever the occasion presented itself. Through all these efforts, however, only a single additional specimen—the *Coenus* from Oklahoma—has been found. Thus it seems evident that both species are rare in nature and that series of specimens likely will not be taken unless especial effort on the part of collectors in general is made. It is in order to call to the attention of such collectors the existence of the species that the writers have decided to characterize them from such short type series.

ALLOPODOPS, NEW GENUS

Oblong-oval, sub-depressed above, moderately convex beneath. Head shorter than pronotum, broader than long, strongly convex, narrowed anteriorly, the sides with a small tooth in front of eyes, then sharply sinuate inwards, jugae rather flat, widest at distal third, not attaining apex of tylus. Eyes prominent, somewhat pedunculate. Ocelli situated behind a line drawn through hind margin of eyes. Antennae quadriarticulate, segment I not attaining apex of head; antenniferous tubercles moderately prominent, visible from above, their outer margin produced much as in related genera. Bucculae reaching practically to base of head, gradually but distinctly elevated posteriorly, their ends almost truncate. Rostrum long, extending to middle of venter. Pronotum with a wide, shallow, transverse depression across its middle and a somewhat narrower one immediately behind the raised anterior margin; the side margins wide, deplanate and somewhat reflexed, triangularly produced at the anterior angle, straight and indistinctly serrulate for the length of the anterior lobe, ending abruptly in front of humeri; the hind margin truncate in front of the scutellum. Scutellum not quite attaining apex of abdomen, broadly rounded distally, the sides parallel along basal fourth. Femora unarmed; tibiae faintly sulcate along upper surface; tarsi tri-articulate. Abdomen beneath with neither median furrow nor opaque areas; connexival segments not spinose.

This genus is erected for the species described below. From related Podopidae it is distinguished by the jugae, which are shorter than the tylus and not swollen, the four-segmented antennae, the expanded and non-interrupted pronotal margins and the absence of a conspicuous metasternal carina.

Allopodops mississippiensis, n. sp.

PLATE I

Black, the rostrum and tarsi piceous-brown, the corium and scutellum along sides and distally somewhat brownish; coarsely and regularly punctate, with a whitish hair arising from each puncture, the hairs long and semi-erect on pronotum and scutellum, elsewhere, shorter, finer, and prostrate. Antennae with apical segment strongly swollen from the base; proportion of segments, 7:9:7:13. Rostrum with segment I not exceeding bucculae, II enlarged from the base, rather strongly compressed laterally, extending to middle of mesosternum, III attaining base of abdomen; proportion, 15:22:18:17.

Scutellum with an oblique impression on each side behind the base, thus leaving a basal triangular portion somewhat raised, the apex of the triangle continued to the middle of disc as a smooth, faint, median carina. Venter with fifth segment (visible fourth) deeply angularly emarginate behind, the last segment at the median line one-half longer than at the sides.

Length, 5.03 mm. Width across humeri, 2.50 mm.; across abdomen, 2.86 mm.

Holotype: female, Wiggins, Mississippi, April 25, 1931, H. G. Johnston.

Coenus inermis, n. sp.

Much larger and slightly broader proportionally than *C. delius* (Say), the coloration and punctuation about as in that species. Head as in *C. delius*, the punctuation, however, noticeably finer than that of protonum. Antennae not suddenly and conspicuously darkened distally; proportions, 16:17:30:25:34. Rostrum darkened distally, extending between hind coxae, segment II in length equal to corresponding segment in *delius*. Pronotum with an indistinct transverse impression on disc in front as in *delius*, the front lateral angle not produced as a small tooth, the humeri more broadly, evenly rounded in male than in female (that of latter is as in *delius*), the lateral margin almost straight, impunctate, as seen from the side the marginal edge much stouter and more obtuse than in that species. Scutellum slightly but distinctly longer than corium, the latter with apex broadly obtusely rounded and much narrower than apex of former. Membrane with veins prominent, embrowned, and irregularly anastomosing. Legs as in *delius*. Venter strongly convex, the connexivum with a brown spot at the basal angle of each segment. Hind margin of genital segment of male evenly rounded, without triangular tooth at the middle.

Length, 11-11.6 mm.; width, 6.5-6.8 mm.

Holotype, male, Marion County, Arkansas, May 7, 1897, F. M. McElfresh; *allotype*, female, McCurtain County, Oklahoma, June 10, 1931, C. C. Deonier; *Paratypes*, two females, taken with holotype.

PLATE I

Allopodops mississippiensis, n. sp.

PLATE I



THE GENERA AND GENOTYPES OF TINGITOIDEA OF THE WESTERN HEMISPHERE

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The superfamily Tingitoidea is represented in the world by over eleven hundred known species, which are divided at the present time among one hundred and forty-four genera. Fifty-two genera, including approximately 424 species and 14 varieties, have been recorded from the Western Hemisphere. When one considers the vast amount of territory as yet untouched by hemipterists this is undoubtedly a rather poor representation of the total number of species existing in the Americas. The only country in South America in which any amount of collecting has been done is Brazil, and its resources are by no means exhausted. There are a few countries in that continent as well as in Insular and Central America where very little or no collecting at all has been done, so it is only logical to suppose that there are a number of new genera and many species yet to be found and described.

This paper catalogues the genera and genotypes of Tingitoidea recorded from the Western Hemisphere. The authors have attempted to make the list complete up to the present time and will appreciate any corrections or additions which may be made. The names of the genera printed in bold face type are arranged alphabetically under subfamilies, and no attempt has been made to show relationship or position. Following the name of the genus is the name of the man who first described it, and on the next line the date and publication in which the description first appeared. The italicized genotype is given in the third line followed by the classification of the type, in parentheses. The definitions for the genotypes used are: haplotype, only species described in the genus at the time of its erection; orthotype, a type by original designation of the author; and logotype, a type by subsequent designation. In cases where no previous designation of type could be found and more than one species was included by the author at the time the genus was erected, the authors have taken it upon themselves to designate a type which will conform to the present status of the genus. These types are labeled "present designation."

Genera which are not valid (synonyms and homonyms) are placed in brackets with the valid genus indicated. In cases where the name is preoccupied by a genus in another order this is also cited. For example, "[*Leptostyla* Stål, 1873; not Lioy, 1864, *Diptera*] (= *Gelchossa* Kirkaldy, 1904) = *Leptopharsa* Stål," means that *Leptostyla*, a genus of *Diptera* founded in 1864 by Lioy, is valid, whereas, *Gelchossa* Kirkaldy, 1904, and *Leptostyla* Stål, 1873, for which *Gelchossa* was erected, later were both suppressed as synonyms of *Leptopharsa* Stål. The note below each valid genus gives the number of described species (from the Western and sometimes also from the Eastern Hemisphere) and other data of importance.

In the near future the authors are planning to publish a check list of the species of Tingitoidea of the Western Hemisphere and are very anxious to secure as many records as possible. Specimens for determination, accompanied by notations of localities and host plants, will be gratefully received and acknowledged.

SUPERFAMILY TINGITOIDEA

Family **PIESMIDAE**

Type of family: Genus *Piesma* Le Peletier and Serville, 1825.

Note: The family Piesmidae contains only two genera, namely, *Piesma* Le Peletier and Serville and *Mcateella* Drake. The latter is known only from Australia.

[*Agrammodes* Uhler] = *Piesma* Le Peletier and Serville.

1895, COLO. EXP. STA. BULL. 31, *Tech. ser.* 1, p. 56.

Type: *Agrammodes costatus* Uhler. (Haplotype).

[*Aspidotoma* Curtis] = *Piesma* Le Peletier and Serville.

1833, ENT. MAG., I, p. 196.

Type: *Aspidotoma (Acanthia) capitata* (Wolff). (Haplotype).

Piesma Le Peletier and Serville (= *Agrammodes* Uhler) (= *Zosmerus* and *Zosnanus* Laporte) (= *Zosmerus* Burmeister) (= *Aspidotoma* Curtis).

1825, ENCYC. MÉTH., X, p. 652 (as subg. of *Tingis*).

Note: Nine species and two varieties described from the Western Hemisphere; many species known from the Eastern Hemisphere. *Agrammodes* Uhler founded upon short-winged form of *Piesma*.

[*Zosmenus* and *Zosnanus* Laporte] = *Piesma* Le Peletier and Serville.

1832, ESS. CLASSIF. SYST. HEMIP. p. 49. (*Zosnanus* on p. 47).

Type: *Zosmenus maculatus* Laporte. (Haplotype).

[*Zosmerus* Burmeister] = *Piesma* Le Peletier and Serville.

1835, HANDB. ENT., II, p. 262.

Note: Probably typographical error for *Zosmenus* Laporte.

Family **TINGITIDAE**

Type of family: Genus *Tingis* Fabricius, 1803.

Note: The family Tingitidae is represented by 3 subfamilies and 51 genera in the Western Hemisphere. Many genera and two subfamilies (or divisions) from the Eastern Hemisphere are not represented in the Americas and, conversely, numerous genera described from the Western Hemisphere are peculiar to the Americas.

Subfamily **CANTACADERINAE**

Type of subfamily: Genus *Cantacader* Amyot and Serville, 1843.

Eocader Drake and Hambleton.

1934, REV. DE ENT., RIO DE JANEIRO, IV, fasc. 4, p. 436.

Type: *Eocader vegrandis* Drake and Hambleton. (Haplotype).

Note: Only one species, described from Brazil.

Nectocader Drake.

1928, IOWA STATE COLL. JOUR. SCI., III, p. 41.

Type: *Nectocader (Cantacader) gounellei* (Drake). (Orthotype).

Note: Two species, both from South America; the descriptions of *N. tingitoides* (Spinola), 1852, and *N. germainii* (Signoret), 1863, were based upon male and female, respectively, of the same species. All South American species described as *Cantacader* have been referred to *Nectocader*.

Phatnoma Fieber.

1844, ENT. MON., pp. 30 and 57.

Type: *Phatnoma laciniata* Fieber. (Haplotype).

Note: Four species and one variety, Central and South America; numerous other species described from the Eastern Hemisphere.

Subfamily **SERENTHIINAE**

Type of subfamily: Genus *Serenthia* Spinola, 1837.

Note: This subfamily is represented in the Western Hemisphere by only two genera.

Coleopterodes Philippi (= *Solenostoma* Signoret, 1863, not Rafinesque, 1815, *Pisces*).

1864, STETT. ENT. ZEIT., XXV, p. 306.

Type: *Coleopterodes (Solenostoma) liliputiana* (Signoret). (Orthotype).

Note: One species, found in South America. *C. fuscescens* Philippi, the monobasic type of *Coleopterodes*, is a synonym of *liliputiana*; (Drake, 1922, Fla. Ent., V, p. 50).

Opisthochasis Berg.

1833, ANN. SOC. ARG., XVI, p. 83; Reprint, 1884, HEMIP. ARG., p. 99.

Type: *Opisthochasis albocostata* (Berg). (Haplotype).

Note: Contains only genotype from South America.

[*Solenostoma* Signoret, not Rafinesque, 1815] = **Coleopterodes** Philippi.

1863, ANN. SOC. ENT. FRANCE, Ser. 4, III, p. 575.

Type: *Solenostoma liliputiana* Signoret. (Haplotype).

Subfamily **TINGITINAE**

Type of subfamily: Genus *Tingis* Fabricius, 1803.

Note: This subfamily contains most of the genera and species of Tingitidae of the world. It is represented in the Western Hemisphere by 45 genera.

Acalypta Westwood (= *Orthostira* Fieb.) (= *Drakella* Bergroth) (= *Fenestrella* Osborn and Drake.)

1840, INTROD. MOD. CLASS. INS., II, Synop., p. 121.

Type: *Acalypta (Tingis) carinata* (Panzer). (Orthotype).

Note: Ten species from the Western Hemisphere, one of which is probably a synonym. Many species from other parts of the world.

Acanthochila Stål.

1861, RIO HEMIP., I, p. 61 (*Acanthocheila*).

Type: *Acanthochila* (*Monanthia*) *armigera* (Stål). (Present designation).

Note: Nine species, all from the Western Hemisphere. Stål. ENUM. HEMIP., III, pp. 119 and 127, changes spelling to *Acanthochila*.

Acysta Champion.

1898, BIOL. CENTR.-AMER., RHYNCH., II, p. 46.

Type: *Acysta integra* Champion. (Logotype).

Note: Seven species and one variety, from Central and South America.

Aepycysta Drake and Bondar.

1932, BOL. MUS. NAC., VIII, pp. 93-94.

Type: *Aepycysta undosa* Drake and Bondar. (Haplotype).

Note: Two species found in Brazil. *Galeatus schwarzi* Drake belongs to this genus.

Allotingis Drake.

1930, BULL. BROOK. ENT. SOC., XXV, p. 269.

Type: *Allotingis* (*Leptobyrssa*) *binotata* (Drake). (Haplotype).

Note: One species, Cuba.

Alveotingis Osborn and Drake.

1916, OHIO STATE UNIV. BULL., XX, p. 245.

Type: *Alveotingis grossocera* Osborn and Drake. (Orthotype).

Note: Three species, all from North America.

Amblystira Stål.

1873, ENUM. HEMIP., III, pp. 119 and 129.

Type: *Amblystira* (*Monanthia*) *pallipes* (Stål). (Haplotype).

Note: Ten species from Central America and South America.

Atheas Champion.

1898, BIOL. CENTR.-AMER., RHYNCH., II, p. 44.

Type: *Atheas nigricornis* Champion. (Logotype).

Note: Ten species, all from the Western Hemisphere.

Australotingis Hacker.

1927, MEM. QUEENS. MUS., IX, p. 29.

Type: *Australotingis franzeni* Hacker. (Haplotype).

Note: Two species, one from South America and the other from Australia.

[Cadamustus Distant] = Stephanitis Stål.

1903, ANN. SOC. ENT. BELG., XLVII, p. 47.

Type: *Cadamustus typicus* Distant. (Present designation).

[Cadmilos Distant] = Galeatus Curtis.

1909, ANN. SOC. ENT. BELG., LIII, p. 113.

Type: *Cadmilos retarius* Distant. (Haplotype).

Caloloma Drake and Bruner.

1923-24, MEM. SOC. CUB. HIST. NAT., VI, p. 152.

Type: *Caloloma uhleri* Drake and Bruner. (Haplotype).

Note: One species from Lesser Antilles.

Calotingis Drake (= *Neopachycysta* Hacker).

1918, BULL. BROOK. ENT. SOC., XIII, p. 86.

Type: *Calotingis knighti* Drake. (Haplotype).

Note: One species from Texas and one from Australia.

Campylotingis Drake and Bondar.

1932, BOL. MUS. NAC., RIO DE JANEIRO, VIII, p. 89.

Type: *Campylotingis (Tigava) mollicula* (Drake). (Orthotype).

Note: Seven species, South America.

Corycera Drake.

1922, MEM. CARN. MUS., IX, p. 368.

Type: *Corycera comptula* Drake. (Orthotype).

Note: Three species, Brazil.

Corythaica Stål.

1873, ENUM. HEMIP., III, pp. 120 and 128.

Type: *Corythaica (Tingis) monacha* (Stål). (Haplotype).

Note: Seven species, present in only the Western Hemisphere. Several synonyms will be discussed in a forthcoming paper.

Corythucha Stål.

1873, ENUM. HEMIP., III, p. 119 and p. 122.

Type: *Corythucha (Tingis) fuscigera* (Stål). (Logotype).Note: Sixty-three species and five varieties from the Americas; one species from the Philippines has been referred to this genus. Three or four of the North American species probably represent synonyms. *Corythucha* often erroneously spelled with one "h" (*Corythuca*).**Dichocysta** Champion.

1898, BIOL. CENTR.-AMER., RHYNCH., II, p. 33.

Type: *Dichocysta pictipes* Champion. (Haplotype).

Note: One species from North and Central America.

[**Dictyla** Stål] = *Monanthia* Le Peletier and Serville.

1874, ÖFV. VET.-AK. FÖRH., p. 57.

Type: *Monanthia platyomia* Fieber. (Haplotype).**Dicysta** Champion.

1897, BIOL. CENTR.-AMER., RHYNCH., II, p. 5.

Type: *Dicysta vitrea* Champion. (Haplotype).

Note: Six species from Central and South America.

Dolichocysta Champion.

1898, TR. ENT. SOC. LONDON, p. 56.

Type: *Dolichocysta venusta* Champion. (Haplotype).Note: Four North and Central American species, one of which should be placed in synonymy. This genus is very closely allied to *Corythaica* Stål.[**Drakella** Bergroth] = *Acalypta* Westwood.

1922, ANN. SOC. BELG., LXII, p. 152.

Type: *Drakella* (*Fenestrella*) *ovata* (Osborn and Drake). (Haplotype).

Note: Proposed for *Fenestrella*, preoccupied.

Eotingis Scudder.

1890, REP. U. S. GEOL. SURV. TERR., XIII, p. 359.

Type: *Eotingis antennata* Scudder. (Haplotype).

Note: One species, fossil from Florissant, Colo.

Eurypharsa Stål.

1873, ENUM. HEMIP., III, pp. 122 and 133.

Type: *Eurypharsa* (*Tingis*) *nobilis* (Guerin). (Haplotype).

Note: Five species found in Central and South America.

[*Fenestrella* Osborn and Drake] = *Acalypta* Westwood.

1916, OHIO STATE UNIV. BULL. XX, p. 222.

Type: *Fenestrella ovata* Osborn and Drake. (Haplotype).

Note: Bergroth (1922, ANN. SOC. ENT. BELG., LXII, p. 152) points out that this name is preoccupied and proposes the name *Drakella*.

Galeatus Curtis (= *Cadmilos* Distant).

1833, ENT. MAG., I, p. 196.

Type: *Galeatus spinifrons* Fallen. (Haplotype).

Note: Two species from North America. Many species found in Eastern Hemisphere.

Gargaphia Stål.

1873, ENUM. HEMIP. III, pp. 119 and 124.

Type: *Gargaphia* (*Monanthia*) *patricia* (Stål). (Logotype).

Note: Thirty-six species, all from the Western Hemisphere.

[*Gelchossa* Kirkaldy] (= *Leptostyla* Stål. not Lioy 1864, *Diptera*) = *Lep-
topharsa* Stål.

1904, ENTOMOLOGIST, XXXVII, p. 280.

Type: *Gelchossa* (*Tingis*) *oblonga* (Say). (Orthotype).

[*Hanuala* Kirkaldy] = subg. of *Leptodictya* Stål.

1905, BULL. SOC. ENT. FRANCE, 15, p. 217.

Type: *Hanuala leinakhoni* Kirkaldy. (Haplotype).

Note: Subgenus of *Leptodictya* Stål; see Drake (1931).

Hesperotingis Parshley.

1917, PSYCHE, XXIV, p. 21.

Type: *Hesperotingis antennata* Parshley. (Orthotype).

Note: Seven species and two varieties, all from North America.

Leptobyrsa Stål.

1873, ENUM. HEMIP., III, pp. 119 and 123.

Type: *Leptobyrsa* (*Tingis*) *steini* (Stål). (Haplotype).

Note: Of the 17 species all but 2 Australian species were described from Central and South America. This genus needs to be revised and either subgenera or new genera erected for some of the species.

Leptocysta Stål.

1873, ENUM. HEMIP., III, pp. 122 and 127.

Type: *Leptocysta* (*Tingis*) *sex-nebulosa* (Stål). (Haplotype).

Note: Two species, both from South America.

Leptodictya Stål.

1873, ENUM. HEMIP., III, pp. 121 and 127.

Type: *Leptodictya* (*Monanthia*) *ochropa* (Stål). (Logotype).

Note: Drake (1931, BOL. MUS. NAC., VII, p. 120), divides genus into 2 subgenera, *Leptodictya* and *Hanuala* Kirk., the former represented by a single South American species and the latter by 26 American and one Japanese species. The generic position of the Japanese species should be verified.

Leptopharsa Stål (= Leptostyla Stål, 1873; not Liroy, 1864, Diptera) (= Gelchossa Kirkaldy, 1904).

1873, ENUM. HEMIP., III, pp. 122 and 126.

Type: *Leptopharsa elegantula* Stål. (Logotype).

Note: Kirkaldy (1904) proposes *Gelchossa* as a new name for *Leptostyla*; Drake (1928) makes *Gelchossa* a synonym of *Leptopharsa*. Contains 52 American species and a few from other parts of the world. The genus *Leptopharsa* badly needs revision and should be divided into two or three subgenera or genera. Most of the described and many undescribed species are found in Central and South America. Drake and Hambleton have the descriptions of many new Brazilian species in press.

[Leptostyla Stål, 1873; not Liroy, 1864, Diptera] (= Gelchossa Kirkaldy, 1904) = Leptopharsa Stål.

1873, ENUM. HEMIP., III, pp. 120 and 125.

Type: *Leptostyla* (*Tingis*) *oblonga* (Say). (Logotype).

Leptoypha Stål.

1873, ENUM. HEMIP., III, pp. 121 and 129.

Type: *Leptoypha* (*Tingis*) *mutica* (Say). (Haplotype).

Note: Eleven American and one Asiatic species.

Liotingis Drake.

1930, BULL. BROOK. ENT. SOC., XXV, p. 270.

Type: *Liotingis evidensis* Drake. (Haplotype).

Note: Two Brazilian species.

Macrotingis Champion.

1897, BIOL. CENTR.-AMER., RHYNCH., II, p. 22.

Type: *Macrotingis biseriata* Champion. (Present designation).

Note: Two species and one variety from Central America.

[Maecenas Kirkaldy] = Stephanitis Stål.

1904, ENTOMOLOGIST, XXXVII, p. 280.

Note: Proposes *Maecenas* for *Tingis* Lethierry and Severin.

Megalocysta Champion.

1897, BIOL. CENTR.-AMER., RHYNCH., II, p. 5.

Type: *Megalocysta pellucida* Champion. (Haplotype).

Note: Three species from South and Central America.

Melanorhopala Stål.

1873, ENUM. HEMIP., III, p. 130.

Type: *Melanorhopala clavata* Stål. (Present designation.)

Note: Four North American species.

[*Mokanna* Distant] = *Stephanitis* Stål.

1910, FAUN. BRIT. IND., RHYNCH., V, p. 111.

Type: *Mokanna princeps* Distant. (Haplotype).

Monanthia Le Peletier and Serville (= *Dictyla* Stål, 1874).

1825, ENCYC. MÉTH., X, p. 653.

Type: *Monanthia (Tingis) rotunda* (Herrich-Schaeffer). (Logotype).

Note: Eleven species from America and approximately three times as many from the Eastern Hemisphere. Distant (1902, ANN. NAT. HIST., Ser. 7, IX, p. 357) treats *M. lanceolata* Walker from Brazil as non-existent and states that the type is not in the British Museum. Many species of tingitids were wrongly described as members of the genera *Monanthia* and *Tingis* and later transferred to other genera or new genera erected for them.

[*Neopachycysta* Hacker] = *Calotingis* Drake.

1928, MEM. QUEENS. MUS., IX, p. 183.

Type: *Neopachycysta subopaca* Hacker. (Haplotype).

Note: Hacker (1929, MEM. QUEENS. MUS., IX, p. 334) made this genus a synonym of *Calotingis* Drake.

Neotingis Drake.

1922, MEM. CARN. MUS., IX, p. 366.

Type: *Neotingis hollandi* Drake. (Haplotype).

Note: Single species from Brazil.

Nyctotingis Drake.

1922, MEM. CARN. MUS., IX, p. 362.

Type: *Nyctotingis osborni* Drake. (Haplotype).

Note: Single Brazilian species.

[*Orthosteira* Fieber] = *Acalypta* Westwood.

1844, ENT. MON., p. 46.

Type: *Orthosteira (Tingis) cassidea* (Fallen) = *Acalypta musci* (Schrank). (Present designation).

Note: Fieber (1861, EUR. HEM., pp. 36 and 130) changes spelling to *Orthostira*.

Pachycysta Champion.

1898, TR. ENT. SOC., LONDON, p. 59.

Type: *Pachycysta diaphana* Champion. (Orthotype).

Note: Three species from South and Central America.

[*Phyllochisme* Kirkaldy] = *Physatochila* Fieber.

1904, ENTOMOLOGIST, XXXVII, p. 280.

Note: Proposes *Phyllochisme* for *Physatochila* Lethierry and Severin.

Physatocheila Fieber (= *Phyllochisme* Kirkaldy, 1904).

1844, ENT. MON., p. 80.

Type: *Physatocheila quadrimaculata* Wolff. (Logotype).

Note: Six described North American species, one or two of which

should be treated as varieties or placed in synonymy. Many species described from the Eastern Hemisphere.

Pseudacysta Blatchley.

1926, *HETER. EAST. N. AMER.*, p. 497.

Type: *Pseudacysta (Acysta) perseae* (Heidemann). (Haplotype).

Note: Represented by a single species from Southern United States and Mexico.

Sphaerocysta Stål.

1873, *ENUM. HEMIP.*, III, pp. 120 and 128.

Type: *Sphaerocysta (Tingis) globifera* (Stål). (Logotype).

Note: Six species and one variety from South America.

Stenocysta Champion.

1897, *BIOL. CENTR.-AMER., RHYNCH.*, II, p. 28.

Type: *Stenocysta pilosa* Champion. (Haplotype).

Note: Two species from Central and South America.

Stephanitis Stål (= *Cadamustus* Distant, 1903) (= *Maecenas* Kirkaldy, 1904) (= *Mokanna* Dist., 1910) (= *Tingis* Lap., 1832 not Fabr.).

1873, *ENUM. HEMIP.*, III, pp. 119 and 123.

Type: *Stephanitis (Tingis) pyri* (Fabricius). (Logotype).

Note: Four species from North and South America; many species in the Eastern Hemisphere. Two species, *S. pyrioides* and *S. rhododendri*, are common to both hemispheres. Horvath (1906, makes *Cadamustus* Distant and *Maecenas* Kirkaldy synonyms of *Stephanitis*. Horvath (1912) divides the genus *Stephanitis* into subgenera *Mendora* (*formosa* Horvath, type; present designation), *Stephanitis* (*pyri* Fabr., type; present designation), *Norba* (*mendica* Horvath, type; present designation), and *Omoplax* (*desecta* Horvath, type; present designation). The American species all belong to the subgenus *Stephanitis*. In his monograph of the genus, Horvath failed to include *S. mitrata* Stål from South America.

[*Taphrostethus* Fieber] = **Cantacader** Le Pelletier and Serville.

1844, *ENT. MON.*, p. 40.

Type: *Taphrostethus quinquecostatus* Fieber. (Haplotype).

Teleonemia Costa.

1865, *ANN. MUS. ZOOL. NAP.*, II, p. 144.

Type: *Teleonemia funerea* Costa. (Logotype).

Note: Forty-three species and one variety described from the Western Hemisphere, a few of which are synonyms. Numerous species from the Eastern Hemisphere which have been described as *Teleonemia* do not all belong to this genus. The subgenera *Americia* Stål, *Amaurosterphus* Stål, and *Teleonemia* Costa are not now treated as divisions of the genus *Teleonemia*.

Tigava Stål.

1860, *RIO. HEM.*, I, p. 63.

Type: *Tigava praececellens* Stål. (Haplotype).

Note: Seven species from South and Central America and one from Australia.

Tingis Fabricius.1803, **SYST. RHYN.**, p. 124.Type: *Tingis (Cimex) cardui* (Linnaeus). (Logotype).Note: Five species from Western Hemisphere; many others from the Eastern Hemisphere. This genus is divided into the subgenera *Tingis* Stål, *Lasiotropis* Stål, *Tropidocheila* Fieb., *Caenotingis* Drake and *Birgitta* Lindberg.**Zatingis** Drake.1928, **IOWA STATE COLL. JOUR SCI.**, III, p. 44.Type: *Zatingis extraria* Drake. (Haplotype).

Note: Single species from Paraguay.

THE MANUFACTURE OF BLUE CHEESE (ROQUEFORT TYPE) FROM HOMOGENIZED COWS' MILK¹

(PRELIMINARY REPORT)

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The manufacture of blue cheese (roquefort type) from cows' milk in the United States has not been entirely successful. Manufacturers have met with various difficulties, among which are: (a) The failure of the mold to develop properly in the cheese; (b) the yellow color of the cheese, especially when made during the period of luxuriant pastures; (c) the failure of the cheese to develop enough of the characteristic roquefort flavor in a reasonable length of time; and (d) the lack of uniformity in successive lots of cheese. More cheese manufacturers would probably attempt to make a blue cheese if a procedure could be developed which would tend to eliminate some of these difficulties.

Currie (1) found that the sharp, peppery flavor, characteristic of roquefort cheese, is due in large part to the accumulation of caproic, caprylic, and capric acids and their easily hydrolyzable salts during the ripening of the cheese. Presumably, these products result from the hydrolysis of some of the fat by lipolytic enzymes of the milk and the normal mold of the cheese.

From the general effect of homogenization on the flavor of raw milk, it appears that the homogenization of milk for blue cheese would be a logical procedure in attempting to bring about a comparatively rapid formation of fatty acids, since the process would enormously increase the surface area of the fat globules and thus facilitate the action of lipolytic enzymes.

COMPARISON OF NORMAL AND HOMOGENIZED MILK

A number of experimental lots of blue cheese were made in which normal and homogenized milk were compared. Usually about 50 gallons of fresh, whole milk were thoroughly mixed, standardized to about 4.0 per cent fat and a portion of it homogenized at 2,000 pounds pressure, with the temperature between 90° and 100° F. The two portions of milk, normal and homogenized, were placed in small cheese vats and the normal milk was made into blue cheese, using the method described by Goss, Nielsen, and Mortensen (2) for the manufacture of Iowa blue cheese except that the mold powder was mixed with the curd before hooping rather than dusted on the curd in the hoops.

With the homogenized milk it was generally necessary to modify the cheesemaking procedure since the curd formed was comparatively soft and brittle and, if handled in the same manner as the curd from normal

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milk, would result in an undesirable, soggy cheese unsuited for the growth of mold. The modifications were: (a) The firming time was usually extended about 30 minutes, and the curd was heated to from 90° to 92° F. by the addition of hot whey; (b) the removal of the curd from the vat to the drain cloth and the mixing in of the mold powder, draining, and hooping were completed in a relatively short time, usually only 5 to 10 minutes being required to complete all of these procedures; and (c) the hoops were turned frequently (every 15 minutes) for about 2 hours.

GENERAL EFFECT OF HOMOGENIZATION

Differences between the curd made from homogenized milk and that made from normal milk were regularly noted during and immediately following the manufacturing process. The curd from homogenized milk appeared to be more flaky and decidedly less colored than that of the control cheese; this color difference was also apparent on the outside of the freshly made cheese. The fresh curd made from homogenized milk was commonly characterized by a rancid taste and odor, due presumably to the action of the inherent lipase of raw milk on the finely divided fat.

After only a few weeks of ripening, the cheese made from homogenized milk regularly began to develop a flavor resembling that of roquefort type cheese, although there was more of a butyric acid flavor than is normally found in typical roquefort, while in the control cheese nothing but the usual, unpleasant, bitter flavor was apparent. At this time the mold growth in the homogenized milk cheese was commonly more luxuriant than that in the control and was brilliant green in color, while the growth in the control cheese was gray green. This contrast in the intensity of mold coloration may have been due, in part, to the color difference in the curd of the two types of cheese.

After from two to three months, the cheese made from homogenized milk had lost the flavor suggestive of butyric acid and had developed considerable of the sharp, peppery flavor characteristic of roquefort type cheese, although the flavor was not as pronounced as that of a fully ripened cheese. No typical roquefort flavor had developed in the control cheese at this time and the bitter flavor was still evident. Throughout the ripening, the cheese made from homogenized milk was characterized by a soft, flaky body, a desirable light color, and a brilliant colored mold growth, while the control cheese was comparatively hard and yellow in color. Three to four months of ripening were necessary before any roquefort flavor could be detected in the control cheese.

COMMERCIAL LOTS OF CHEESE FROM HOMOGENIZED MILK

Commercial lots of blue cheese were made from homogenized milk to determine the possibilities of the method developed. Usually about 700 pounds of milk were homogenized and made into 16 cheese weighing about 5 pounds each. Examinations of the cheese from time to time indicated that the general character of the cheese obtained was the same as with the small lots of homogenized milk.

DISCUSSION

The results indicate that the manufacture of blue cheese from homogenized cows' milk, rather than from milk not homogenized, offers certain

possibilities from the standpoints of curing and marketing. From the studies completed, it appears that the cheese made from homogenized milk ripens in a relatively short time and regularly has a white color comparable with the French cheese made from sheep's milk; in addition, the body is unusually soft so that the cheese may be readily spread with a knife. At present, there is no information available on the keeping qualities of the cheese made from homogenized milk since none has been held for extended periods. Additional studies on the manufacture and the biological aspects of this cheese are now in progress.

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STUDIES ON THE INCUBATION OF THE CHINCH BUG EGG¹

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Incubation periods of various lengths have been reported for the eggs of the chinch bug, *Blissus leucopterus* (Say). Forbes (1895) reported the incubation period as varying from 12 to 22 days and Stedman (1902) found the eggs to begin hatching in the field in about 16 days. Headlee and McCulloch (1913) made observations on the incubation period of 63 eggs and found the shortest time to be 9.6 days and the longest 18 days. Luginbill (1922) reported the incubation period of 664 eggs as varying from 9 to 31 days and found a difference of nine days in the time of hatching in the same lot of eggs. He found 8 per cent infertility in one lot of 503 eggs. Flint and Larrimer (1926) observed eggs to hatch in from 7 to 45 days, apparently depending upon the temperature. Shelford (1932) made observations on 34 eggs under various combinations of controlled temperature and relative humidity and found the incubation period to range from 3 to 20 days. This lower limit of 3 days was considerably shorter than any of the periods observed by the present authors as well as much briefer than those reported by the other investigators mentioned.

These variations in the incubation period have probably resulted largely from the influence of such environmental factors as temperature and relative humidity. Studies were conducted during the summer of 1935 for the purpose of determining the duration of the incubation period and the percentage of hatch under various conditions of constant temperature and constant relative humidity. The temperatures employed were 19.5° C., 24.5° C., 29.5° C. and 34.5° C. Temperature variations did not exceed $\pm 0.5^\circ$ C., except at 19.5° C. in which case the variation was approximately $\pm 1.0^\circ$ C. The relative humidities were 20 per cent, 40 per cent, 60 per cent, 80 per cent and 100 per cent. In addition one lot of eggs was incubated on wet blotting paper kept saturated with distilled water. A total of 2,400 eggs was employed, 100 at each combination of temperature and relative humidity. The eggs were deposited in cotton plugs at the base of young growing wheat plants in the rearing cages. In each cage there were confined from three to five pairs of adult bugs in order that fertilization should be assured insofar as possible. At four-hour intervals the cotton plugs were removed and the eggs contained therein used for the experiments. The eggs were selected at random, except that obviously injured eggs were not used. In this way an attempt was made to have them as much alike as possible, with a difference in age of not more than four hours.

At the time when the eggs were hatching, readings were taken at four-hour intervals, and the duration of the incubation period taken as the time from the middle of the four-hour interval at the end of which the

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TABLE 1. *Ranges of incubation periods, 50 per cent hatch-points and percentage of hatch of 100 chinch bug eggs under various conditions*

| Temperature | Percentages of relative humidity | | | | In contact with water |
|---|--|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 |
| 19.5° C. Range 50 per cent hatch-point Hatch | * | * | 852-904 hrs. 864 hrs. 35 per cent | 756-868 hrs. 784 hrs. 72 per cent | 604-736 hrs. 668 hrs. 83 per cent |
| 24.5° C. Range 50 per cent hatch-point Hatch | 400-432 hrs. 416 hrs. 5 per cent | 372-412 hrs. 394 hrs. 63 per cent | 348-400 hrs. 372 hrs. 70 per cent | 348-380 hrs. 369 hrs. 85 per cent | 324-276 hrs. 350 hrs. 72 per cent |
| 29.5° C. Range 50 per cent hatch-point Hatch | 276-280 hrs. 276 hrs. 4 per cent | 252-288 hrs. 272 hrs. 34 per cent | 236-280 hrs. 251 hrs. 62 per cent | 216-260 hrs. 232 hrs. 98 per cent | 200-232 hrs. 212 hrs. 84 per cent |
| 34.5° C. Range 50 per cent hatch-point Hatch | ** 1 per cent | 164-200 hrs. 176 hrs. 62 per cent | 164-196 hrs. 170 hrs. 87 per cent | 156-192 hrs. 165 hrs. 92 per cent | 152-184 hrs. 159 hrs. 87 per cent |

* No eggs hatched under these conditions.

** Only one of the 100 eggs hatched; this between 190 and 294 hours.

eggs were collected, to the middle of the four-hour interval at the end of which they were found to have hatched, plus or minus two hours.

Table 1 summarizes the lengths of the incubation periods by giving the limits of the time during which hatching occurred at each combination of temperature and humidity. It also gives the time required for approximately half of the eggs to hatch, and the percentage of eggs which hatched in each lot of 100 at each combination of temperature and humidity.

It is evident from these tables that the greatest effect of temperature was upon the length of the incubation period, whereas relative humidity affected chiefly the percentage of hatch. The incubation periods at the various temperatures were roughly as follows: 30 days at 19.5° C., 15 days at 24.5° C., 10 days at 24.5° C. and 7 days at 34.5° C.

INCUBATION AT 19.5° C.

A total of 600 eggs was incubated at 19.5° C. Six different relative humidity levels were maintained as indicated above. The length of the incubation period at each humidity is shown in table 1. The shortest time was 604 hours, approximately 25 days. This record was obtained at 100 per cent relative humidity. The longest incubation period occurred at 60 per cent relative humidity; this was 904 hours, approximately 38 days. It may be seen by reference to the table that in each case there was an appreciable spread in the time of hatching in the same lot of eggs. Eggs maintained in contact with water began to hatch after 656 hours or about 27 days of incubation. Approximately half of the viable eggs hatched in 29 days. All had hatched after 736 hours, or about 31 days. At 100 per cent relative humidity, 83 eggs hatched over a period of 132 hours. This was the maximum spread encountered in the entire experiment.

Table 1 summarizes the percentage of hatch at each relative humidity. None of the eggs hatched at 20 per cent humidity. At 40 per cent, three nymphs began to hatch, but died while in the process of extricating themselves from the egg shell. Beginning with a hatch of 35 eggs out of the lot of 100 incubated at 60 per cent relative humidity, there was an increase at 80 per cent humidity and a still further increase at 100 per cent humidity. In the latter case, 83 eggs hatched. Only 55 of the eggs hatched out of the lot which was in contact with water.

INCUBATION AT 24.5° C.

The incubation at 24.5° C. was roughly 15 days (table 1). The shortest time was 324 hours or 13 days and the longest, 432 hours or 18 days. These results occurred, respectively, for eggs maintained in contact with water and at 20 per cent relative humidity. At 60 per cent relative humidity there was a period of 52 hours between the time the first egg hatched until all had hatched. There was also a spread of 52 hours in the time of hatching period of the lot of eggs in contact with water.

Table 1 includes a summary of the percentage of hatch at each humidity. A total of 85 eggs hatched out of the lot of 100 at 80 per cent humidity. The numbers which hatched at 60 per cent relative humidity and 100 per cent relative humidity and in contact with water were approximately the same. At 40 per cent humidity the hatch had dropped to 63 eggs and at 20 per cent relative humidity, only 5 eggs hatched.

INCUBATION AT 29.5° C.

Table 1 summarizes the incubation periods of 600 eggs maintained at 29.5° C. The shortest time recorded was 200 hours or approximately 8 days, and the longest 288 hours or 12 days. It may be seen by referring to the table that the relative humidity seemed to have but little effect upon the incubation period. The greatest spread in the period of hatching in the same lot of eggs occurred at 100 per cent relative humidity, in which case the eggs hatched over a period of 60 hours.

The percentage of eggs which hatched at each humidity is shown in table 1. At 80 per cent humidity 98 eggs hatched out of the lot of 100. This was the highest percentage of hatch encountered throughout the entire experiment. Only four eggs hatched at 20 per cent relative humidity. The number increased to 34 at 40 per cent humidity and to 62 at 60 per cent relative humidity.

INCUBATION AT 34.5° C.

It may be seen by reference to table 1 that the average length of the incubation period at 34.5° C. was 7 days. The minimum incubation period occurred at 100 per cent relative humidity. The time was 144 hours or 6 days. The maximum time was 200 hours or approximately 8 days. This period occurred at 40 per cent humidity. The greatest spread in the time of hatching in the same lot of eggs was 36 hours. This occurred at both 80 per cent and 40 per cent humidity. If the time at which approximately half of the viable eggs hatched at each humidity is considered, it may be seen (table 1) that at this temperature the incubation period was slightly shortened in each case as the amount of moisture increased.

A summary of the percentage hatch at each humidity is included in table 1. Starting with a hatch of one egg at 20 per cent humidity, the number of eggs which hatched continually increased with an increase in the percentage of relative humidity until at 80 per cent 92 eggs hatched. Beyond this point the number decreased. Only 87 eggs hatched at 100 per cent relative humidity and 81 hatched out of the lot in contact with water.

The influence of temperature upon the time required for incubation is illustrated in figure 1, which shows also that temperature exercises a fairly uniform influence on the length of time which elapses before any hatching takes place, as well as on the spread of the period during which hatching occurs at each set of environmental conditions.

Figure 2 shows the spread in the time during which hatching took place in two typical cases. These two lots of eggs were maintained at 34.5° C. and at respective relative humidities of 40 per cent and 80 per cent. In every case there was considerable spread in the length of the incubation period of a given lot of eggs subjected to the same humidity condition. The maximum spread occurred at 19.5° C. and 100 per cent relative humidity, in which case 83 out of a lot of 100 eggs hatched over a period of 132 hours. At the same relative humidity but at 34.5° C., 83 eggs out of a lot of 100 hatched over a period of only 32 hours.

Figure 3 illustrates the effect of humidity by showing the percentage hatch in each lot of 100 eggs at the various relative humidities in each temperature group. It may be seen that at 80 per cent relative humidity at 29.5° C., 98 eggs hatched out of a lot of 100. This was the highest per-

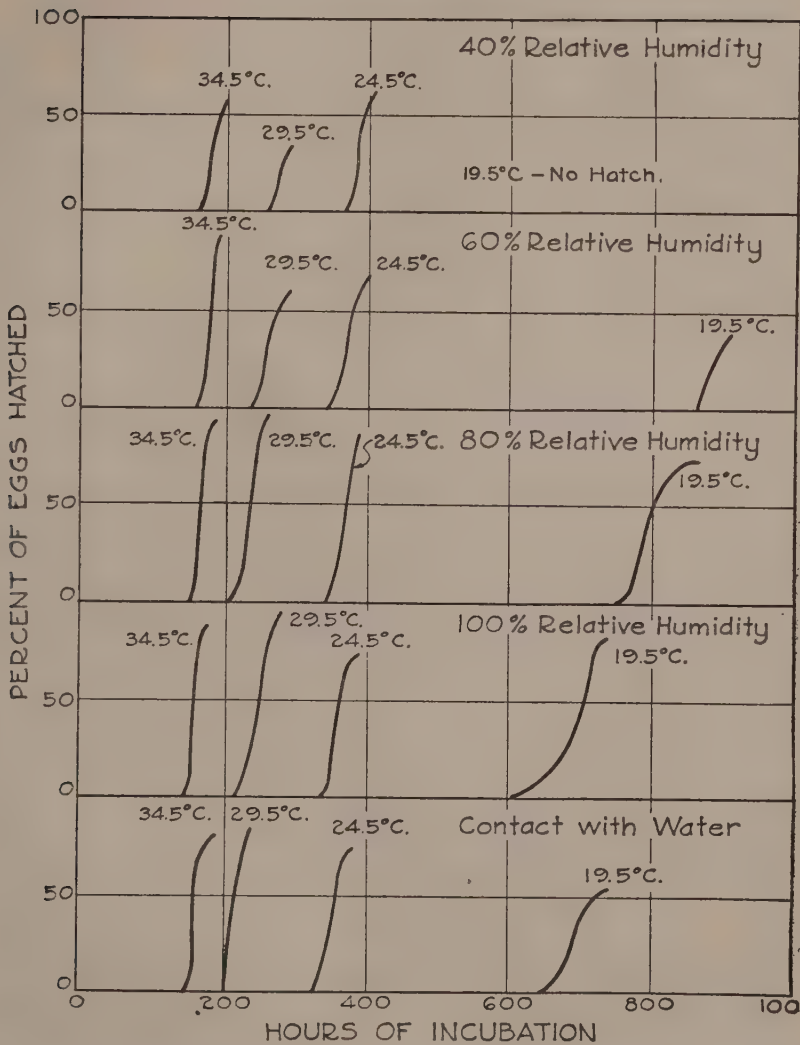


Fig. 1. The relation of temperature to the length of the incubation period of eggs of *Blissus leucopterus*.

centage of hatch encountered. At 19.5° C. none of the eggs hatched at either 20 per cent relative humidity or at 40 per cent relative humidity. At the three higher temperatures the greatest percentage of hatch occurred in each case at a relative humidity of 80 per cent. The number of eggs which hatched at either 60 or 100 per cent relative humidity was somewhat lower than at 80 per cent. Below 60 per cent there was a rapid

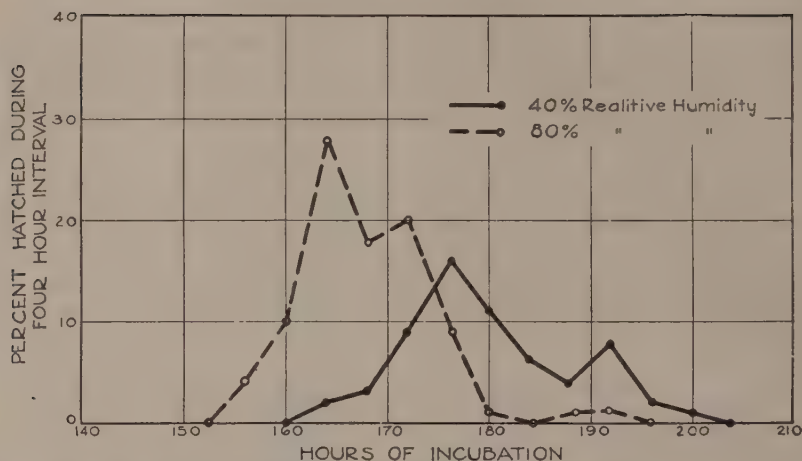


Fig. 2. Hatching time of *Blissus leucopterus* eggs incubated at 34.5° C.

decrease in the number of eggs which hatched until at 20 per cent relative humidity the greatest hatch was only 5 eggs out of 100.

In addition to the experiments described above, 4,100 eggs in various stages of development were collected from the field. It was found that 70.02 per cent of these field eggs hatched.

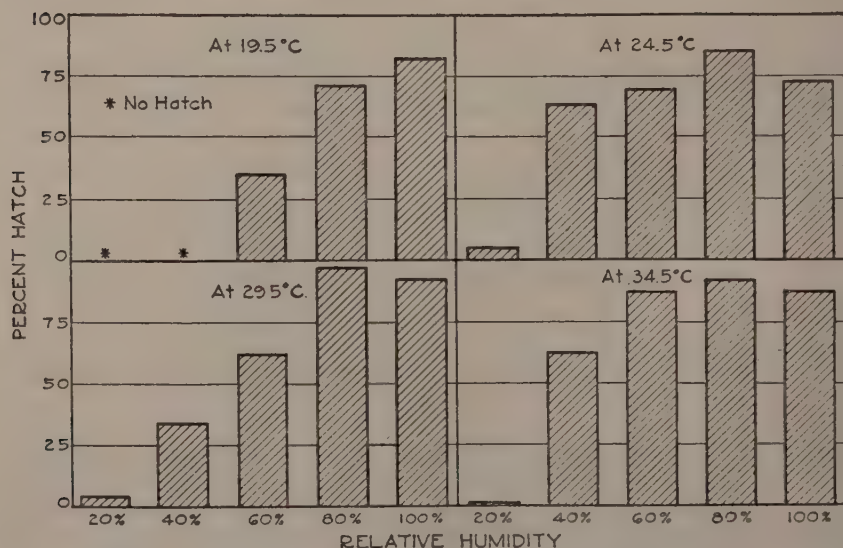


Fig. 3. Effects of humidity upon percentage of hatch of eggs of *Blissus leucopterus*

EXPERIMENTS ON SUBMERGENCE

As an addition to these studies some preliminary experiments were conducted to determine what effect submergence in water might have on the incubating eggs. Several hundred eggs were employed at temperatures of 24.5° C., 29.5° C., and 34.5° C. When not actually submerged the eggs were maintained at 80 per cent relative humidity. In all cases the eggs were found to withstand submergence for considerable periods of time. Eggs kept at 24.5° C. hatched after 10 or 12 days of continuous submergence with only slight decrease in the percentage of hatch. Eggs hatched at this temperature even after 15 days of submergence.

At 29.5° C. 8 separate lots of eggs were used. One lot was submerged immediately. The second was allowed to incubate for one day before being submerged. The remaining lots were submerged after incubating for periods varying by one day, until the last lot had undergone 7 days of incubation. In each lot 10 eggs were removed from the water after 4 hours of submergence, and 10 more at each 4 hour interval thereafter until all were removed. In this way eggs of various ages were allowed to undergo submergence for periods varying from 4 hours to 6 days. The percentage of hatch even after 6 days of submergence was only slightly reduced. The age of the eggs at the time that submergence began apparently had little effect upon the number of eggs which hatched in each case.

In experiments on intermittent submergence at 29.5° C. eggs were found to hatch if removed from water only one hour per day.

Eggs maintained at 34.5° C. withstood continuous submergence for 4 days.

Submergence lengthened appreciably the incubation period. The maximum period encountered at 24.5° C. for the controls was 18 days. A lot of 10 newly laid eggs submerged for 14 days at 24.5° C. began to hatch on the 20th day and by the 22nd day a total of 7 eggs had hatched. In another lot of 10 newly laid eggs submerged for 15 days at 24.5° C. 3 eggs hatched on the 22nd day and one hatched on the 24th day. Submergence also lengthened the period of incubation at the higher temperatures employed. There was a fairly direct relationship between the length of submergence and the increase in length of the incubation period.

SUMMARY

Eggs of the chinch bug, *Blissus leucopterus* (Say) hatched in approximately 30 days at 19.5° C., 15 days at 24.5° C., 10 days at 29.5° C. and 7 days at 34.5° C.

Temperature seemed not to affect appreciably the percentage of hatch.

In each case there was considerable spread in the time of hatching of a given lot of eggs subjected to the same conditions.

Relative humidity, although apparently influencing the length of the incubation period in some cases to a slight extent, has its greatest effect on the percentage of hatch. The most favorable relative humidity at the higher temperatures was 80 per cent. In one case 98 eggs hatched out of a lot of 100.

Chinch bug eggs hatched after submergence in water for considerable periods of time. For example, a number of eggs hatched after 15 days

of submergence at 24.5° C. At higher temperatures the eggs were less resistant to submergence.

An increase in the incubation period occurred in fairly direct proportion to the period of submergence.

A number of eggs were hatched after being submerged continuously for 23 hours out of each 24 during the period of incubation.

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STUDIES ON TEMPERATURE AND MOISTURE AS FACTORS INFLUENCING WINTER MORTALITY IN ADULT CHINCH BUGS¹

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Overwintering chinch bugs in Iowa and other midwestern states are often subjected to extreme and sudden changes of temperature and moisture conditions during the winter months. More specific knowledge as to the exact effect which either of these factors, alone or the two acting together, has upon the total winter mortality would enable entomologists to obtain a better estimate of the probable surviving or spring population.

Since an exceedingly large number of chinch bugs entered winter quarters in the fall of 1934, it seemed an opportune time to begin a study of this problem. The investigations undertaken included both field observations and laboratory studies, but only the laboratory phases of the problem are discussed in the present paper.

METHODS

All of the chinch bugs used in these experiments were typical long-winged adults of *Blissus leucopterus* (Say). Since holding the bugs under artificial conditions in the laboratory might produce changes in their physiological conditions, it was deemed advisable to secure fresh experimental material from the field for each set of experiments. With the exception of the material collected in Washington County, all bugs used were taken from clumps of bunch grasses, *Andropogon scoparius* Mich. and *Andropogon furcatus* Muhl. Those from Washington County were collected from an area of mixed cover, consisting of various grasses, sticks, leaves, and other debris at the edge of a woodlot.

Unless otherwise indicated, the bugs brought in from the field were very slowly warmed, and then only enough to induce feeble movement so that the living individuals could be recognized. As soon as the bugs began moving about, they were picked up with an aspirator and divided indiscriminately into 50 lots of 10 bugs each, the subsamples of 10 being placed in cotton-stoppered shell vials (20 × 60 mm.).

The samples of 500 bugs each were then exposed to various low temperatures, as indicated in connection with the individual experiments. At the completion of each exposure the insects were kept at a temperature of approximately 22° C. for 2 hours and then examined for living and dead bugs. To check these results, each lot was recounted after a 24-hour interval. In all cases a bug that was able to move its appendages was counted as living.

Refrigerating chambers maintaining constant temperatures of 5.0° C., 0.0° C., - 6.6° C., - 12.2° C., and - 17.7° C. (variation ± 0.5° C.) were

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used in the experiments. In a few instances another unit in which the temperature ranged between -20.0°C . and -25.0°C . was used. In making short exposures, the 50 vials were spread out upon a precooled tray and then placed immediately in the low temperature box so that all the vials would receive the same temperature exposure. Likewise, this method facilitated prompt removal of the vials at the termination of the exposure.

To make gradual exposures, the entire set of 50 vials was placed in a double-walled Cellotex box which was constructed so that a one-half inch space was left between the two layers of cellotex. The actual temperature within the vials was determined by inserting two thermometers through the top of the box in such a way that the bulbs of the thermometers were in two separate vials.

Laboratory experiments in certain instances were checked by comparing 15-minute exposures in the refrigerator with exposures of the same length made at the same temperatures out-of-doors.

In the experiments involving relative humidity, the bugs were counted into the usual lots and placed in shell vials, but cheesecloth coverings held by rubber bands instead of cotton stoppers were used. Sealed glass chambers containing a graduated series of sulfuric acid solutions were used to insure uniform relative humidity conditions. These chambers were then placed in the desired temperature unit and kept there until the completion of the experiment. The special technique used in connection with certain experiments is explained in the discussion accompanying these experiments.

Each time samples were brought in from the field, two lots of 500 bugs were placed at 0.0°C . and 100 per cent relative humidity and kept there as controls until experiments with bugs from that particular sample were completed.

EXPERIMENTAL

SUDDEN SHORT EXPOSURES TO LOW TEMPERATURES

In an effort to determine the effect of sudden short exposures to low temperatures on bugs collected from the same place at different times of the year and those taken from various places at approximately the same time during the winter, over 25,000 chinch bugs were divided into samples of 500 each and exposed to various low temperatures ranging between -7.0°C . and -23.5°C . These overwintering bugs were collected from three widely separated points in Iowa, namely, Des Moines, Washington and DeWitt. The data from these experiments are presented graphically in figure 1.

In all cases temperatures higher than -7.0°C . were not fatal to the bugs at 15-minute exposures. Below -7.0°C ., however, the mortality increased rapidly until at about -15.0°C . it ranged between 70 and 92 per cent.

Below -15.0°C . the increase in mortality is less rapid, indicating that a few of the bugs in each sample were much more resistant to low temperatures than the others. As no controlled low temperature units below -23.0°C . were available, the temperature at which all the bugs would be killed by a 15-minute exposure was not determined. However, from the trend of the curves it is apparent that under the conditions of this ex-

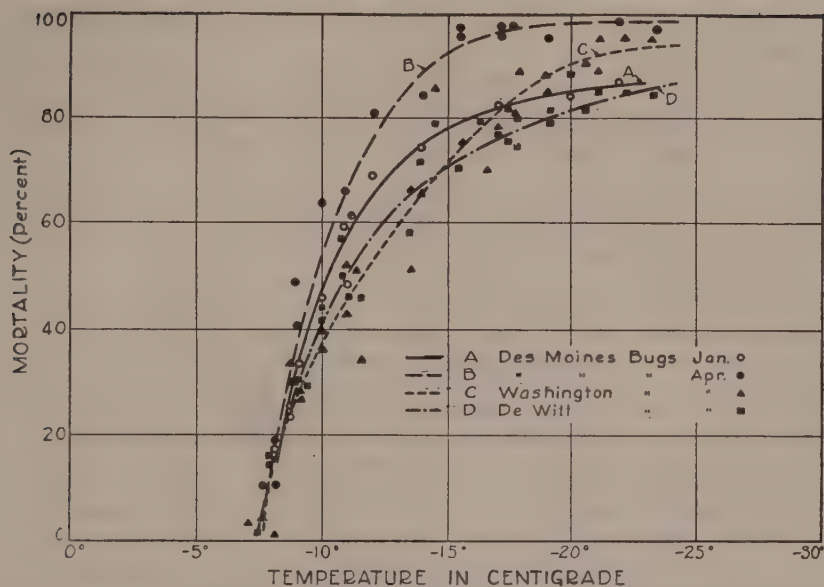


Fig. 1. Mortality of chinch bugs collected at places and times indicated and exposed to various temperatures for 15 minutes.

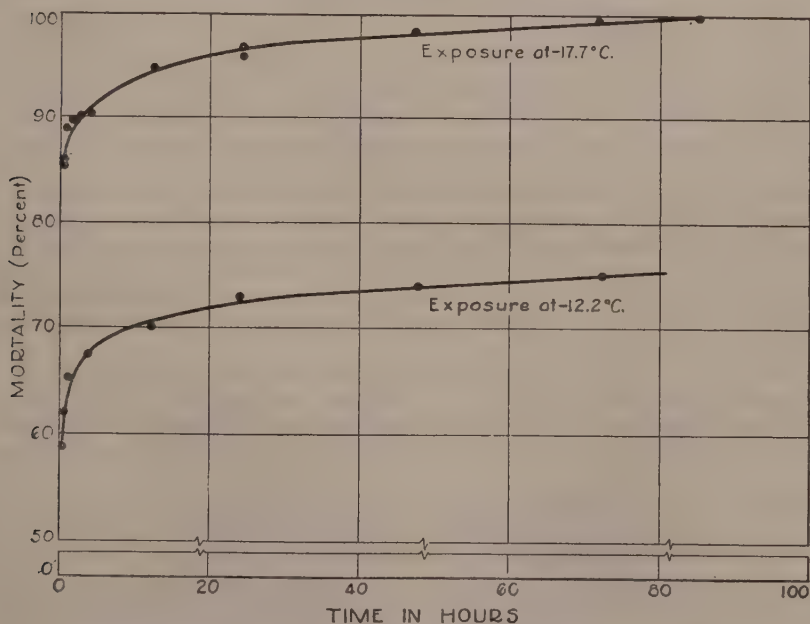


Fig. 2. Mortality of chinch bugs when subjected to prolonged exposures at -12.2°C . and -17.7°C .

periment a temperature of about -25.0°C . would produce a mortality of at least 90 per cent.

Although there is some spread of the points on each curve, the trend of all four curves is the same. Furthermore, it should be noted that the points on curve A fit much more closely to the line than do the points on curves B, C and D. This may be interpreted as indicating that bugs collected early in the season are more uniform than those collected later. Furthermore, the greatest degree of variability is found in the Washington County bugs (curve C) collected late in the season from a variety of habitats within the same immediate vicinity.

The data presented as curves A and B, the latter representing bugs collected at Des Moines, April 1, and the former representing bugs collected during January, show that the two groups differ from each other in their resistance to low temperatures. Statistical tests on these data show the differences are significant. The bugs collected April 1 were fairly active at the time the collection was made, and it is entirely possible that they had imbibed some moisture or plant juices which would account for their greater susceptibility to low temperatures. A comparative study of the curves in figure 1 shows that biotic and seasonal conditions influence to some extent the resistance of the bugs. However, if a zone were established which would include all of these curves, one might select overwintering bugs at random, subject them to low temperatures under the conditions imposed in these experiments, and expect to have the results fall within this zone.

PROLONGED EXPOSURES

To determine the effect of prolonged exposure to low temperatures, chinch bugs collected at Des Moines were placed at constant temperatures of -12.2°C . and -17.7°C . At intervals, lots of 500 bugs were withdrawn from each series, and the average mortality for each sample was determined. The data resulting from this experiment are shown graphically in figure 2. At both temperatures there was a comparatively large increase in mortality for each additional hour of exposure until about the tenth hour, after which the rate of increase in mortality for each hour of exposure was gradually reduced. When mortality is plotted against the logarithm of time (fig. 3), the curves become straight lines and lie almost parallel to each other. At -17.7°C . 100 per cent mortality was reached at the end of 85 hours; at -12.2°C ., however, only 75 per cent of the bugs were dead at the end of 72 hours.

Simultaneously with the initiation of the above experiment, bugs from the same source were subjected to a constant temperature of -6.6°C . In this case, as is shown in table 1, the death rate was comparatively low for the first several days, after which it increased rapidly. It appears, there-

TABLE 1. *Percentage of chinch bugs dead after exposure for various intervals of time at -6.6°C . (0 F.). Five hundred bugs in each test*

| Time exposed (days) | 1 | 2 | 3 | 3.5 | 8 | 13.5 | 16 | 21 | 28 |
|---------------------|-----|-----|-----|-----|------|------|------|------|------|
| Percentage dead | 0.6 | 2.0 | 3.6 | 6.0 | 11.6 | 22.8 | 44.6 | 67.6 | 92.2 |

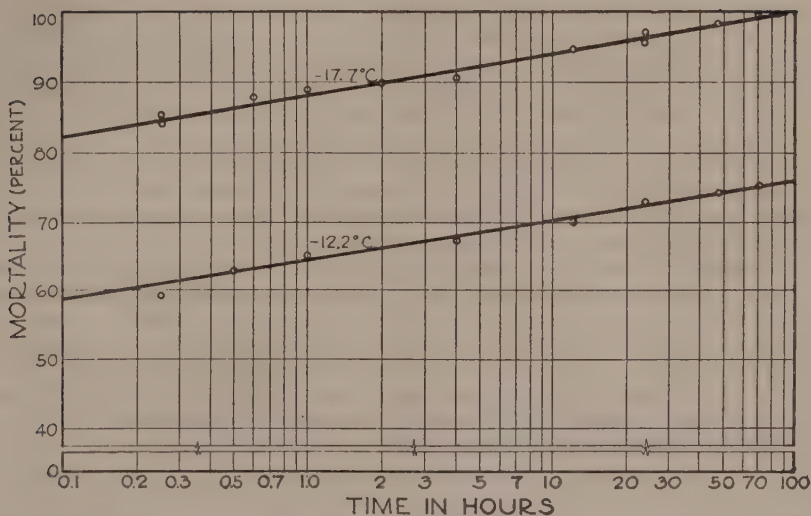


Fig. 3. Mortality of chinch bugs when subjected to prolonged exposures at -12.2°C . and -17.7° . Logarithms of time.

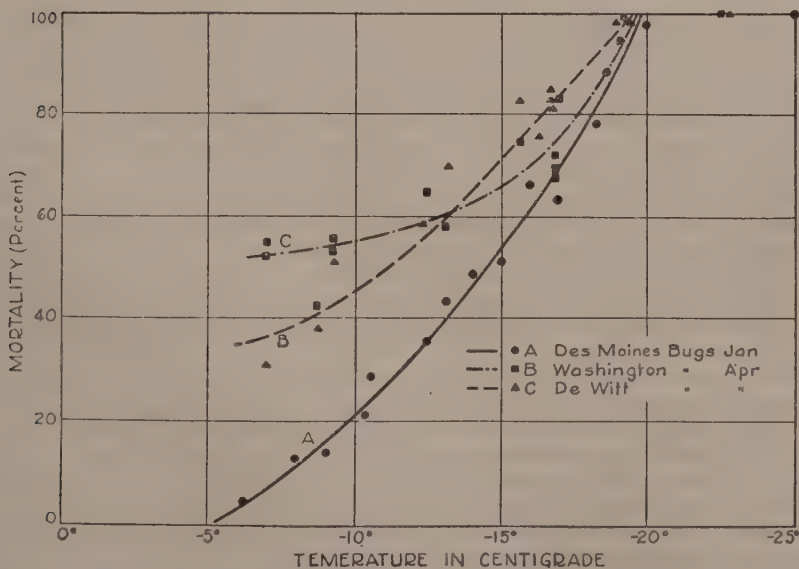


Fig. 4. Mortality of chinch bugs obtained after gradual exposures to various low temperatures.

fore, that the temperature was too high to produce a large initial mortality, and it is possible that death resulted from factors independent of temperature. This thought is suggested by the fact that the bugs from this experiment died with their appendages fully extended; whereas, those involved in experiments with lower temperatures died with their appendages contracted and held more or less tightly against their bodies. After this experiment was completed, some of the surviving bugs were, in several instances, left in the laboratory overnight and then returned to the low temperature unit. Later it was observed that these individuals lived longer than the others in the same experiment. In one case, for example, 7.0 per cent were living at the end of a 35-day period.

Another series of this same group of chinch bugs were wrapped in a wet paper towel and placed at $+4.3^{\circ}\text{C}$. in a container at a relative humidity of 100 per cent and left for a period of 96 days. At the end of this time 876 of the 1,000 bugs originally started were still alive and active. A number of pairs of the surviving bugs were placed on growing wheat plants, and it was observed that they lived longer and produced more eggs than bugs which hibernated under natural field conditions.

Two hundred of these bugs were left in a low temperature unit at $+1.0^{\circ}\text{C}$. from January 3 until August 3, at which time 17 of them were found to be living. When these were given access to wheat seedlings in a constant temperature box at 34.5°C ., mating began almost immediately and egg deposition started August 7.

GRADUAL EXPOSURES

To determine whether or not the chinch bugs' ability to withstand low temperatures might be increased by exposing them to gradually falling temperatures, a series of experiments, using bugs from Des Moines, DeWitt and Washington, Iowa, were conducted. After being held for 24 hours at a temperature of -6.6°C . the bugs were placed in an insulated box and changed to a colder temperature chamber, where the temperature inside the box fell at the rate of 1.5°C . per hour. The results of these experiments are presented graphically in figure 4. The gradual exposure appeared to increase the resistance of the bugs to moderately low temperatures, that is, temperatures ranging from -7.0°C . to -15.0°C . At temperatures between -15°C . and -18°C . the mortalities resulting from gradual and instant exposures were not significantly different from one another, and at temperatures below -20.0°C . the bugs receiving a gradual exposure showed a higher mortality than those exposed for only a short period. This is no doubt at least partially due to the prolonged exposures to moderately low temperatures.

Here, as in the other experiments, bugs collected from different areas and at different times showed wide differences in their responses to the same treatment. It is apparent that bugs collected April 1, after they had become somewhat active, were more easily killed than those collected in mid-winter. No doubt, the imbibition of plant juices or water caused the decrease in their resistance.

STEPPED EXPOSURES

In a somewhat similar experiment, which might be called a stepped exposure, the bugs were placed at -6.6°C . for 24 hours. At the end of

that period they were dropped abruptly to -12.2 or -17.7° C. and exposed to one of those temperatures for varying periods of time.

Here as in the case of the gradual exposures, the precooled bugs showed a definite increase in resistance to short exposures, but as the exposures were prolonged their apparent increased resistance disappeared and they were killed at approximately the same rate as bugs which were not precooled.

Late in the season bugs from another field sample were held for 60 hours at -6.6° C. and then placed at -12.2° C. for varying lengths of time. The results, shown in curve C, fig. 5, seem to indicate that the longer period of precooling greatly increased the resistance of the bugs, but, since the samples were collected at different times, some of this variation might have been due to differences in the bugs.

EXPOSURES OUT-OF-DOORS OVERNIGHT

On nine different nights while this investigation was in progress, samples of 500 bugs were placed out-of-doors in a screened insectary. The following morning they were taken into the laboratory, and after a 2-hour interval the percentages of living bugs were determined. The results which are presented in figure 6 show that here, as in most of the other experiments, death began to occur at about -7.0° C. or -8.0° C. and that all of the bugs were killed at a temperature of -25.0° C. (-13.0° F.).

Three very important factors, namely, the minimum temperature reached, the influence of a gradual exposure and the influence of a prolonged exposure at temperatures slightly higher than the minimum, have a bearing on the trend of the curve plotted from these data. This curve therefore should not be compared with the other curves without a consideration of the modifying influences involved.

FREEZING THE BUGS IN THE ORIGINAL SODS

In all of the experiments discussed so far, the bugs were warmed to induce activity so that the living bugs could be recognized and counted. It was evident that this procedure might be open to serious question. To overcome this objection 25 small clumps of bunch grass containing overwintering chinch bugs were cut in halves and one part of each clump was held at -6.6° C. for 48 hours, while the other half was placed at -17.7° C. for the same period. At the end of the exposure the samples were examined and the numbers of living and dead bugs were recorded.

By applying Abbott's formula $\frac{(x - y) 100}{x}$ to the data thus obtained,

it was possible to determine the mortality directly attributable to the low temperature.

The data accumulating from this experiment show rather conclusively that the chinch bugs subjected to this treatment are little if any more resistant to low temperatures than those which were warmed and counted before freezing (table 2).

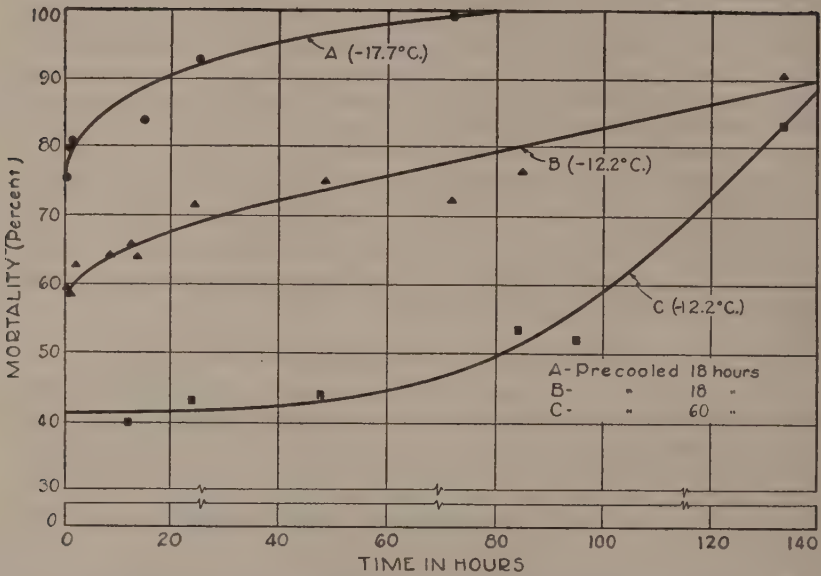


Fig. 5. Mortality of chinch bugs obtained by stepped exposures in which the bugs were precooled and then subjected to the temperatures indicated.

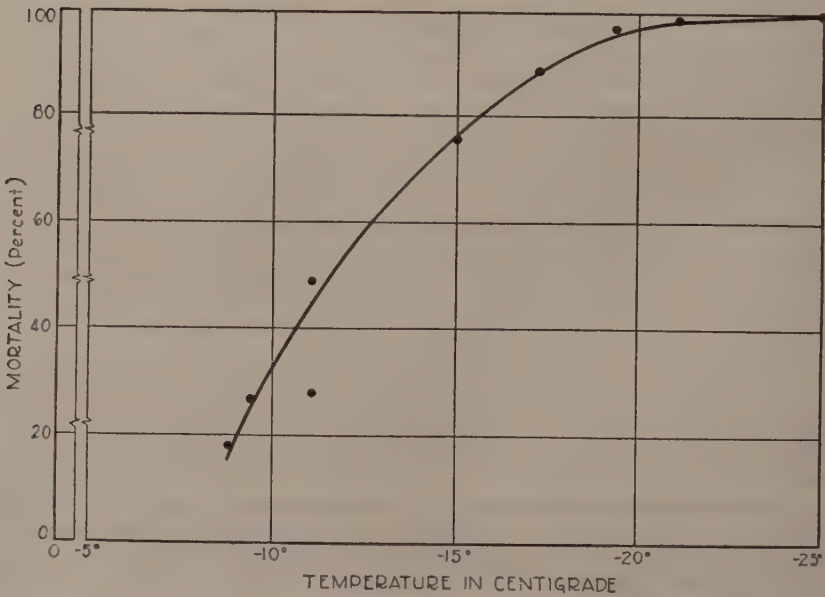


Fig. 6. Mortality of chinch bugs after exposure out-of-doors overnight (temp. = min. temp.).

TABLE 2. *Results of an experiment on freezing chinch bugs at -17.7° C. without removing them from the bunch grass sods*

| Number of samples | Percentage dead in original sample (control) | | | | Percentage dead after 48 hours at -17.7° C. | | | | $x-y^*$ (100) |
|-------------------|--|------|------|------|--|-------|------|------|---------------|
| | No. Bugs | Max. | Min. | Mean | No. Bugs | Max. | Min. | Mean | |
| | | | | | | | | | x |
| 8 | 911 | 75.0 | 30.0 | 49.5 | 782 | 100.0 | 73.4 | 96.9 | 93.8 |
| 5 | 1555 | 73.9 | 4.1 | 49.3 | 1599 | 99.2 | 75.4 | 96.6 | 93.3 |
| 6 | 407 | 55.0 | 13.0 | 35.6 | 289 | 97.0 | 80.0 | 90.6 | 85.3 |
| 3 | 1156 | 40.2 | 23.6 | 30.3 | 1301 | 99.5 | 98.6 | 99.1 | 98.7 |
| 3 | 42 | 69.1 | 30.0 | 41.4 | 40 | 100.0 | 95.0 | 97.5 | 95.8 |

$x - y$ (100)

* $\frac{x - y}{x}$ = percentage of control (where x = number alive in check and y = number alive in experiment).

HOLDING THE BUGS AT HIGH AND LOW RELATIVE HUMIDITIES BEFORE FREEZING

In an effort to determine whether or not the humidity at which the bugs were held previous to exposure to cold conditions greatly altered the resistance of the bugs, over 12,000 individuals were divided into two separate lots, one of which was placed at 0 per cent relative humidity and the other at 100 per cent relative humidity. Those bugs maintained at a relative humidity of 100 per cent were also given access to contact moisture in the form of wet paper toweling. Both groups were held at a constant temperature of 0.0° C.

At regular intervals, 500 bugs were taken from each container and exposed for a 15-minute period at -17.7° C. (0° F.).

The data (table 3) plainly show that beginning with the first 24 hours there is a very significant difference between the two series of bugs. The mortality of the bugs kept at 0 per cent relative humidity varied from 74.0 to 79.4 per cent, and except on the first reading there was a gradual reduction in the percentage of mortality, whereas the percentage of mortality of those bugs kept at 100 per cent relative humidity and contact moisture varied from 86.6 to 90.8 per cent. As a check against this experiment 500 bugs from the original stock culture were exposed to a temperature of -17.7° C. for 15 minutes. In the latter case 82.2 per cent of the bugs were killed. It would appear, therefore, that the loss of water by the

TABLE 3. *Mortality of chinch bugs after a 15-minute exposure at -17.7° C. (0° F.) when the bugs were previously held for various lengths of time at 0° C. and 0 per cent relative humidity or at 0° C. and in contact with moisture. Five hundred bugs in each group*

| Duration of pre-treatments (days) | 1 | 2 | 4 | 6 | 8 | 12 |
|--|------|------|------|------|------|------|
| Percentages dead—held at 0 per cent R. H. | 79.4 | 79.4 | 79.2 | 78.2 | 75.0 | 74.0 |
| Percentages dead—held in contact with moisture | 90.8 | 90.0 | 86.6 | 89.2 | 90.0 | 89.0 |

bugs increased their resistance to low temperatures, and that contact with moisture decreased their resistance.

EFFECT OF DRINKING WATER PREVIOUS TO EXPOSURE TO LOW TEMPERATURES

Having demonstrated that atmospheric humidity and contact moisture influence the ability of chinch bugs to withstand low temperatures, it was considered advisable to extend the experiments. One group of bugs was placed at 0 per cent relative humidity. A second lot was given access to wet paper toweling from which they could imbibe water freely. Both lots were placed at room temperatures (22° C.), and at intervals of 4, 12 and 72 hours, 500 bugs from each group were exposed to a temperature of -17.7° C. for a 15-minute period. The results of these experiments are shown in table 4. A check lot of 500 bugs from the same original stock culture given a similar exposure for 15 minutes showed a mortality of 82.8 per cent.

TABLE 4. *Mortality of chinch bugs after a 15-minute exposure at -17.7° C. (0° F.) when the bugs were previously held for various lengths of time at 22° C. (71.6° F.) and at 0 per cent relative humidity or at 22° C. and in contact with moisture (and allowed to drink). Five hundred bugs in each group*

| Duration of pre-treatments (hours) | 4 | 12 | 72 |
|---|------|------|------|
| Percentage dead—held at 0 per cent R. H. | 77.6 | 87.2 | 84.0 |
| Percentage dead—held in contact with moisture | 88.6 | 95.4 | 93.0 |

Bugs which imbibed water from the saturated towels were more easily killed in every instance. Also it was apparent that the resistance of the bugs was somewhat increased by moderate dehydration, but long exposures to an extremely dry atmosphere at a relatively high temperature may have weakened the bugs. At least it is reasonable to assume that a portion of the increased mortality shown by bugs dehydrated for 12 and 72 hour periods may be due to causes other than low temperatures. This supposition is supported somewhat by the fact that many bugs were dying in the jars at the time the bugs were counted.

To check further the influence of the imbibition of water by the bugs a large number of bugs were given access to water on paper towels for a 12-hour period at room temperature. At the end of this time they were counted into 5 groups of 250 bugs each and given a 15-minute exposure at temperatures ranging from -7.0° C. to -16.0° C. At the same time 5 lots of 500 bugs each, taken from the original stock culture, were given identical exposures. The data (table 5) show rather convincingly that those bugs having access to water were consistently more easily killed than those taken directly from their overwintering quarters.

EFFECT OF RELATIVE HUMIDITY ON SURVIVAL AT MODERATELY LOW TEMPERATURES

At the outset of the experiments it was observed that chinch bugs held for a number of days in an electric (+2° C.) refrigerator showed an ever increasing mortality rate. This indicated that humidity or factors other than temperature were causing death under this condition.

TABLE 5. *Mortalities obtained when chinch bugs given access to water for 12 hours at 22° C., and bugs taken directly from the field were exposed for 15 minutes at the temperatures indicated. Five hundred bugs in each group*

| Temperature in degrees C. | —7.0 | —10.2 | —11.0 | —14.0 | —16.0 |
|---|------|-------|-------|-------|-------|
| Percentage dead—bugs with access to water | 7.0 | 55.8 | 64.0 | 84.0 | 88.8 |
| Percentage dead—bugs direct from field | 1.2 | 45.6 | 53.2 | 70.8 | 74.8 |

In an endeavor to determine the importance of relative humidity as a factor causing death under the above mentioned condition, 600 chinch bugs were counted and divided into 6 groups so that each lot contained 10 shell vials of 10 bugs each. Five of these lots were placed at 0 per cent, 25 per cent, 50 per cent, 75 per cent and 100 per cent relative humidities, respectively. The bugs in the sixth lot were wrapped in moist paper toweling and placed at relative humidity of 100 per cent. All were kept at a temperature of 0.0° C. The vials were removed from their respective jars, and the live and dead bugs recorded at frequent intervals. After each reading the dead bugs were discarded, and the live bugs were returned to their respective jars.

These data (figure 7), it will be observed, show rather conclusively that bugs held at low relative humidities die much sooner than those living in a moist atmosphere. Under the conditions of this experiment the death rate seems to be directly related to the saturation deficiency of the atmosphere.

The above experiment was repeated, but this time the containers were held in a cellar in which the temperature ranged from —2.0° to +4.0° C. The resulting data were comparable to those presented, the only difference being that the slightly higher temperature shortened the survival period.

In connection with these experiments 250 chinch bugs were placed at 0.0 per cent relative humidity and 0.0° C. and held for 15 days, at which time 60 per cent of the bugs were dead. The surviving bugs, which were all in a greatly weakened condition, were wrapped in moist paper toweling and placed at a relative humidity of 100 per cent. Within a week these bugs were seemingly normal and just as active as other bugs that had not been subjected to the dry atmospheric conditions.

EFFECT OF VARIOUS RELATIVE HUMIDITIES UPON WEIGHT LOSS

Concurrent with the last experiment 600 bugs were divided into samples of 100 and subsamples of 10 as before. The bugs in each shell vial were weighed in grams to the fourth decimal place and then the six groups were placed in the various relative humidity chambers and held at a temperature of 0.0° C. At regular intervals the vials were removed and the weight of the bugs was recorded. A few readings were made after the first deaths were noted, but corrections were made by deducting the weight of the dead bugs and computing the percentage loss of weight on the basis of the living bugs only.

A graphic representation of these data is given in figure 8. With a decrease in percentage relative humidity there is a corresponding increase

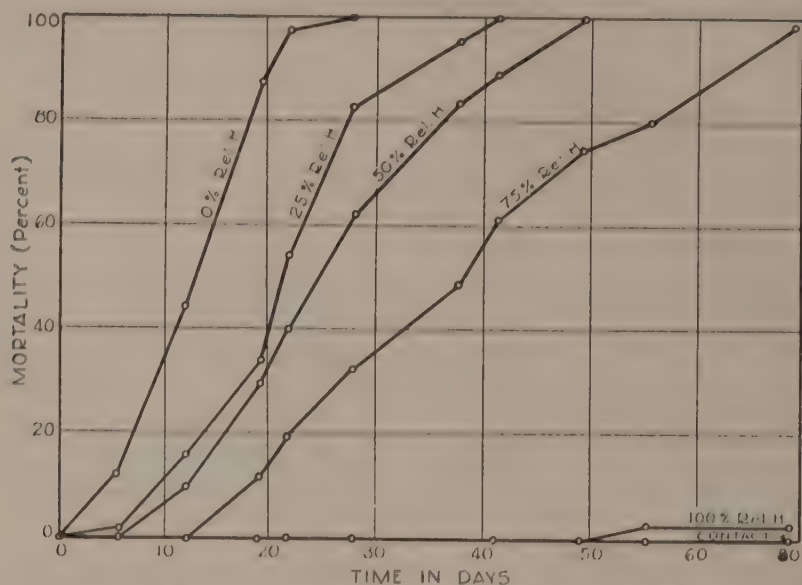


Fig. 7. Mortality of chinch bugs when subjected to various relative humidity conditions at 0.0° C.

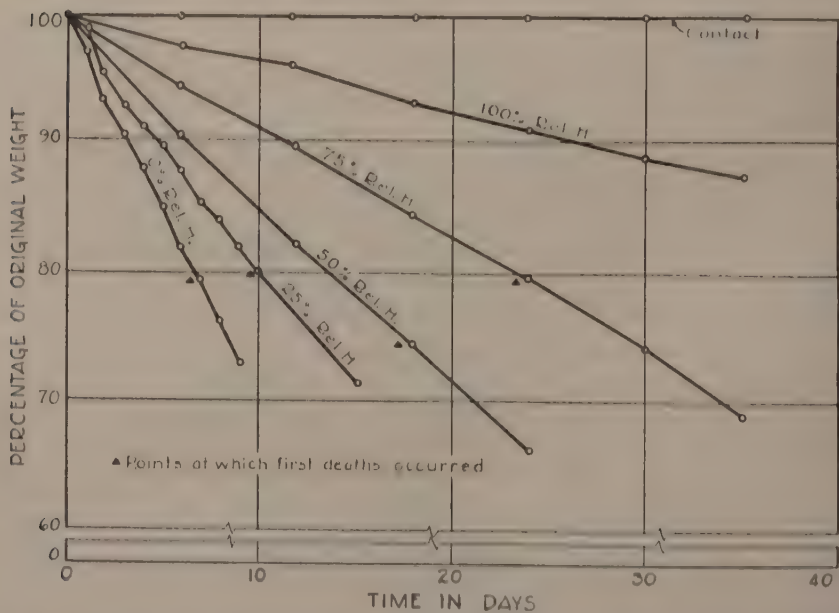


Fig. 8. Loss in weight of chinch bugs when subjected to various relative humidity conditions at 0.0° C.

in the rate of weight loss. Furthermore, it is apparent that for each relative humidity there is a direct relationship between the length of exposure and the percentage loss in weight, and in general this appears to be a straight line relationship. Also, it is obvious that death began to occur when the bugs were reduced to between 75 and 80 per cent of their original weight. In the case of the bugs wrapped in the wet paper towels and kept at a relative humidity of 100 per cent, no loss of weight could be detected at the end of 35 days when the experiment was discontinued.

EFFECT OF SUBMERGENCE ON OVERWINTERING CHINCH BUGS

Grasslands and wooded areas in which chinch bugs often spend the winter are frequently inundated by floods. Britcher (1903) reported that adults could live after being submerged for a 75-hour period, but the temperature at which the experiment was conducted was not given. Janes, Hager and Carman (1934) found that in the summer adult chinch bugs do not survive if immersed longer than 36 hours at 24.5° C. Since no data available deal with submergence during the overwintering period, a study of this phase of the problem seemed desirable.

A number of wire screens (40 meshes to the inch) were made in the form of envelopes, 2 inches wide by 3 inches long. Chinch bugs (25 to each screen) were dropped into these envelopes and the open ends were closed. The screens containing the bugs were immersed in ice water which was held at 0° C. by frequent additions of ice.

Each day an envelope containing 25 chinch bugs was removed from the water, placed on a paper towel, and the numbers of bugs both alive and dead were recorded. These bugs were then placed in vials, and after 24 hours the reading was repeated to make certain that no seemingly dead bugs had recovered, and, conversely, that no more had died. By reference to table 6, it will be observed that even after 22 days 40 per cent of the bugs were still alive. After the first few hours of submergence, the bugs recovered in a short time (about 25 minutes), but as submergence was prolonged it took an increasingly longer time for the bugs to resume activities. After 20 days submergence it was noted that more than 4 hours were required for the bugs to start moving about. It is possible that during submergence basal metabolism continued anaerobically, and un-oxidized metabolites simply accumulated; thus, by the time the chinch

TABLE 6. *Mortalities obtained when chinch bugs were submerged for various lengths of time in ice water (0° C.). Twenty-five bugs in each group*

| Days submerged | Percentage dead | Days submerged | Percentage dead |
|----------------|-----------------|----------------|-----------------|
| 1 | 0 | 12 | 12 |
| 2 | 0 | 13 | 20 |
| 3 | 4 | 14 | 28 |
| 4 | 4 | 15 | 40 |
| 5 | 4 | 16 | 44 |
| 6 | 8 | 17 | 44 |
| 7 | 8 | 18 | 52 |
| 8 | 8 | 19 | 60 |
| 9 | 4 | 20 | 60 |
| 10 | 8 | 21 | 60 |
| 11 | 16 | 22 | 60 |

bugs were again restored to air they were greatly in arrears for oxygen, or, in other words, the bugs had built up an oxygen debt.

In another submergence experiment the bugs were confined in glass tubes (20 mm. \times 60 mm.) which were closed at both ends with a piece of cheesecloth held in place by a rubber band. They were cooled at -6.6° C. and dropped into a jar of 10 per cent sodium chloride solution (freezing point -7.4) which had been previously reduced to the same temperature. After 54 hours submergence, 50 per cent of the bugs were alive. The temperature was then lowered to -8.0° C., at which temperature a mushy ice was formed, and after a 66-hour exposure 42.6 per cent of the bugs were still alive. It is rather surprising that the bugs could withstand such a highly saturated solution of salt for so long a period.

On one occasion a sample of bunch grass which had been continuously under water for about three weeks, in an area on the Wapsipinicon River, was brought into the laboratory, and over 40 per cent of the bugs in the sample were still alive.

EFFECT OF FREEZING IN ICE

In samples of bunch grass taken from the field it was noted that the clumps of grass which were frozen in solid ice always yielded a very high percentage of dead bugs. On numerous occasions pieces of ice, containing masses of frozen bugs, were broken loose and thawed out slowly in a cool room. Living bugs were found only in a few instances. Many of the dead bugs had been crushed and in some cases dismembered.

Britcher (1903) conducted a number of experiments in which submerged bugs were frozen in a block of ice and concluded that "Freezing while submerged in water is almost sure to be fatal." Parks (1934) stated that chinch bugs ". . . can be frozen in wet debris, or even ice, without causing their death," but his accompanying illustration (figure 3) seems to indicate that the water was frozen while the bugs were floating on the surface and that they were not submerged in the water at the time of freezing. The entomological literature contains a number of references to instances in which living chinch bugs have been taken from ice-coated plants, but the circumstances under which the observations were made are not given.

In an endeavor to throw more light upon this subject the following experiments were designed to approach the problem from different angles and, where possible, to approximate field conditions.

Four 4-inch flower pots were filled with moist soil to within two inches of the top and then placed in a room at -6.6° C., where they were held until the soil was frozen. Then 200 chinch bugs, chilled to inactivity, were placed in each flower pot, and a layer of dry bunch grass was spread over the bugs. In two of the pots wet snow was firmly packed over the straw layer and water was added to form a sheet of ice over the top of the snow, thus approximating a sleet storm condition. In the other two pots the layer of snow was omitted, and one and one-half inches of water was poured over the straw and allowed to run down around the bugs and the straw so that all was frozen in a solid mass, approximating the freezing of standing water.

In this latter series of pots, besides the 200 chinch bugs placed under the straw, three additional lots of bugs were placed in the water before it was frozen. These bugs were treated as follows:

First, 25 bugs were placed in 40 mesh, brass screen cylinders, 10 × 60 mm.; second, 50 bugs were placed in steel tubes tightly corked; third, 125 bugs were placed in five 10 mm. gelatine capsules, 25 bugs in each. This entire experiment was repeated 3 times.

As is shown in tables 7 and 8 the bugs frozen in the ice died in every case. Those in wire screens with water around them also died. Whereas, in the flower pots covered with an ice sheet, but with no water in contact with the bugs, only about 20 per cent of the bugs were killed. Those chinch bugs frozen in the gelatine capsules were killed to the extent of 60 per cent, and it should be mentioned here that the capsules which were only partially compressed by the ice contained more live bugs than those that were greatly compressed.

TABLE 7. *Mortalities obtained when chinch bugs were frozen in ice and under an ice sheet for 24 hours at -6.6° C. (20° F.). Two hundred bugs in each group*

| Sample number | I | | II | | III | |
|--|------|------|------|------|------|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Percentage dead when protected by snow cover under ice | 46.5 | 23.5 | 10.0 | 14.0 | 19.0 | 9.0 |
| Percentage dead when frozen in ice | 100 | 100 | 100 | 100 | 100 | 100 |

TABLE 8. *Percentage of mortality obtained when chinch bugs confined in various types of containers were submerged and frozen for 24 hours at -6.6° C. (20° F.)*

| Trial | Sample | Bugs confined in screen cylinder | | | Bugs confined in gelatine capsules | | | Bugs confined in closed steel tube | | |
|-------|--------|----------------------------------|------|------------|------------------------------------|------|------------|------------------------------------|------|------------|
| | | Number | | Pctg. dead | Number | | Pctg. dead | Number | | Pctg. dead |
| | | alive | dead | | alive | dead | | alive | dead | |
| 1 | 1 | 0 | 25 | 100 | 37 | 88 | 70.4 | 44 | 6 | 12.0 |
| | 2 | 0 | 25 | 100 | 55 | 70 | 56.0 | 40 | 0 | 20.0 |
| 2 | 1 | 0 | 25 | 100 | 45 | 80 | 64.0 | 30 | 20 | 40.0 |
| | 2 | 0 | 25 | 100 | 42 | 85 | 68.0 | 44 | 6 | 12.0 |
| 3 | 1 | 0 | 25 | 100 | 43 | 82 | 64.8 | 43 | 7 | 14.0 |
| | 2 | 0 | 25 | 100 | 62 | 73 | 58.4 | 42 | 8 | 16.0 |

Apparently, then, one of the factors responsible for the death of the bugs when frozen in water, under these conditions, is their compression by the stress forces in the ice and not temperature or lack of oxygen. However, lack of oxygen might eventually kill the few bugs not killed by pressure. The survival of the bugs frozen in the brine mixture where the ice was mushy in character lends support to this view.

When clumps of bunch grass sod (approximately 2 inches in diameter) containing overwintering bugs were immersed in water and frozen for a 48-hour period at -6.6° C., the results were somewhat different. Here, as is shown in table 9, only 51.0 to 60.3 per cent of the bugs were killed by freezing in ice at this temperature. It was noted, however, that as the water froze, air bubbles were forced from the stems of the plants,

TABLE 9. *Mortality obtained when chinch bugs in a clump of sod were frozen for 48 hours*

| Sample | Frozen at -6.6°C. | | | Frozen at -17.7°C. | | |
|--------|-----------------------------------|------|------------|------------------------------------|------|------------|
| | Number | | Percentage | Number | | Percentage |
| | alive | dead | | alive | dead | |
| 1 | 61 | 92 | 60.3 | 2 | 106 | 98.1 |
| 2 | 23 | 37 | 61.6 | 3 | 116 | 97.5 |
| 3 | 54 | 59 | 51.0 | 1 | 39 | 97.5 |

from the turf, and from the soil itself, and it seems possible that this reduced the pressure and stresses which might otherwise have developed, thus somewhat decreasing the mortality. In this experiment several bugs which floated out of the turf were subsequently frozen into the ice and some of these survived. When another series of sods from the same source were submerged in water and frozen for 48 hours at -17.7°C. , the mortality was found to be much higher, ranging from 97.5 to 98.1 per cent dead (table 9). In this case the low temperature was undoubtedly responsible for the increased mortality.

Samples of sod which were partially imbedded in ice formed by the freezing of standing water yielded a high percentage of dead bugs in every case; whereas, other parts of the same sod, which were comparatively dry and free from ice, often yielded a larger percentage of live bugs.

Sods coated with ice as the result of a sleet storm usually yielded a comparatively high percentage of live bugs. In this case it is not improbable that the gradual building of an ice sheet from the group up eliminated much of the pressure which would have resulted from the freezing of standing water.

DISCUSSION

The data presented show that the ability of chinch bugs to withstand freezing temperatures is comparatively low and that the minimum temperatures normally recorded for Iowa and other "Corn Belt" states usually are sufficiently low to produce practically a 100 per cent mortality of unprotected overwintering bugs.

Under field conditions the insulation afforded by an accumulation of dead grass, leaf mulch and snow cover is therefore an important factor in checking winter mortality. The importance of insulation in modifying the effect of freezing temperatures has been previously demonstrated by Mail (1930), Holmquist (1931) and others, all of whom have worked with insects other than the chinch bug.

The environmental conditions surrounding a chinch bug seem to have an important influence upon its ability to withstand low temperatures. Within certain limits, dehydration increases the resistance of the bugs, and, conversely, the imbibition of water shortly before exposure decreases their resistance.

The formation of an ice sheet usually means death to those chinch bugs which are frozen into the solid ice, but chinch bugs below the ice sheet might be affected in either of two ways: (1) If water below the film

of ice drained away leaving an air space, insects in the turf below might benefit by the formation of the dead air space above them; (2) if water fills all or most of the air spaces in the leaf mulch or other natural protection and then freezes into ice, the insulating value of the mulch might be greatly reduced.

Factors other than temperature and humidity may also have an important bearing upon the winter hardiness of the chinch bug. Paine (1929) and Grossman (1931) have presented some evidence which indicates that undernourished insects may be less resistant to low temperatures than normal individuals. It is also possible, though not demonstrated, that variations in the length of adult life prior to entering hibernation might influence the winter hardiness of the bugs.

SUMMARY

1. Chinch bugs collected from different sources varied in their resistance to low temperature exposures.

2. Bugs secured from the same locality at various times during the winter showed considerable variation in susceptibility to cold.

3. When bugs were exposed for a long period at a constant temperature of -12.2 or -17.7°C. , the percentage of mortality increased rapidly for about the first 10 hours and after that the increase was less rapid.

4. Precooling at sublethal temperatures increased the bugs' resistance to temperatures between -7 and -15°C. , but the mortality resulting from exposures to lower temperatures was not significantly different from that obtained by instant exposures.

5. When the temperatures were lowered by steps, a definite increase in resistance to fairly low temperatures (-7 to 12°C.) was noted, but this effect was gradually overcome as the exposure was prolonged.

6. Dehydration produced by brief exposures at low relative humidities increased the resistance of the bugs.

7. Chinch bugs which drank water were less resistant to low temperatures than those which did not.

8. At 0°C. bugs held at high relative humidities lived longer and lost less weight than bugs held at low relative humidities.

9. Chinch bugs submerged in water at 0.0°C. showed a 60 per cent mortality after a 22-day period.

10. Freezing chinch bugs in solid ice proved to be fatal in most instances.

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THE BIOLOGY OF PSEUDOSINELLA VIOLENTA (FOLSOM), WITH
SOME EFFECTS OF TEMPERATURE AND HUMIDITY ON ITS LIFE
STAGES (COLLEMBOLA: ENTOMOBRYIDAE)

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Pseudosinella violenta (Folsom), one of the commonest springtails in the vicinity of Ames, Iowa, occurs under rocks, logs, rotting leaves, in ant nests and in the greenhouses where moisture content of the soil is high. When uncovered in its natural habitat, this white eyeless springtail becomes greatly agitated and darts about from place to place, stopping and suddenly starting again. Because of this activity it has been referred to as the "Scooter" (Folsom, 1924). It ranges throughout the United States, having been recorded from Arkansas, California, Illinois, Indiana, Louisiana, Massachusetts, Minnesota, New York, Tennessee, Texas, Utah and Washington (Mills, 1934).

ECONOMIC IMPORTANCE

Spencer and Stracener (1929, 1930) found that this insect does considerable damage to sugar cane in Louisiana by making pits in the roots and by gnawing off practically all of the side roots, and thereby retarding cane-sprout growth by fourteen per cent in approximately nine weeks. The presence of an abundance of humus in the test cages did not prevent injury to the sugar cane roots. They found also that this insect "causes a marked reduction in growth and final weight and a slight reduction in percentage of sucrose."

Ingram (1931) observed *P. violenta* (Folsom) "grazing" upon root hairs of the cane plant. In his experiments this springtail multiplied very rapidly on a diet restricted to newly cut cane stalks, and a population of ten increased to two hundred fifteen in seven and one-half weeks. An original colony of ten, when fed on soaked corn, multiplied to 3,437 in a period of eight and one-half weeks, and in another experiment, where four parched peanuts served as the only source of food, to 3,554 in seven and one-half weeks.

During his experiments Ingram observed the springtails feeding upon a fungus which grew on the peanuts. In the writers' experiments, however, the fungus growths were not eaten or damaged in any way. The peanuts were first pitted and eventually consumed, but if any portion of them was covered with fungus, that part was left untouched. The results were identical when wheat was used as a food.

Folsom (1933) concludes that "Collembola are of minor importance but are more important than is generally known." He lists *P. violenta* as one of the forty springtails which are known to be injurious. An individual springtail does not cause great harm, but the combined efforts of many of them tend to have a pronounced effect on the plant as a whole.

TECHNIQUE USED IN REARING

Since this insect is very small and active and requires a high amount of relative humidity, it was necessary to use special containers for rearing and observing it. In one method tried specimens were placed in short lengths of glass tubing, the ends of which were plugged with cellu-cotton. This was not satisfactory because moisture tended to condense on the inside of the tubing, resulting in the drowning of the insects. Cotton was substituted for cellu-cotton but it was found that the springtails would become entangled in the fibers and break their antennae and legs.

Thereafter, the following and more successful technique was used throughout the tests. A short piece of glass tubing one-half inch in diameter was covered on one end with silk bolting cloth held in place by a rubber band. A cork was used in the opposite end. Soil and the insects were enclosed and the vial numbers were written directly on the corks. An improvised aspirator or suction apparatus was found best for capturing the insects and transferring them from one vial to another. The aspirator was bent and enlarged in a flame. The bend prevented the insect's returning through the nozzle when it was turned down into a vial or on to the soil, while the bulbous portion acted as a reservoir when many were captured (figure 1).



Fig. 1. The modified aspirator used in handling the living springtails. Note the silk bolting cloth covering the end of the glass tubing is held in place by the rubber tubing.

Eggs were obtained by placing from ten to twenty springtails in a vial containing soil from their natural habitat. These insects were removed the next day and the eggs were left in the soil in the same vial or were sorted into other vials with a camel's hair brush under a binocular microscope.

The vials were supported over solutions of potassium hydroxide of predetermined strengths in closed moisture chambers in order to obtain the desired relative humidities (Buxton, 1931). The moisture chambers were kept in constant temperature cabinets and were removed only for the short intervals necessary for making observations.

REACTIONS TO ENVIRONMENT

When these insects are uncovered in their native habitat they are extremely agitated and do not stop until they are under some projecting piece of soil or other object. This action leads one to suspect that negative phototropism is a strong factor in producing responses. However, the writers' studies convinced them that positive thigmotropism is a greater factor. It was discovered accidentally that they would retreat under a microscope cover glass and appear as contented and as quiet as if they were under an object which would reduce the amount of light. When the springtails were in glass vials where there was soil they preferred to stay close to the source of light. If nothing was provided for them to use as a retreat they would run about very energetically and they would not live long.

The springtails would not stay in contact with one another. If one would get too close to another the antennae were used to strike the intruder away.

The only external sexual differentiation found in this insect was the shape of the genital aperture. That of the male was rounded and was situated on a setiferous biscuit-shaped papilla, while the female opening was merely a transverse slit which had enlarged anterior and posterior lips free of setae. These characteristics could be seen only in cleared specimens and by using a compound microscope. Copulation was not observed at any time.

One hundred offspring of five females were examined and only thirty-five proved to be males. One hundred individuals in the adult instar (head width .033 mm.) were taken at random in the insectary greenhouse and then confined in the same vial for forty-eight hours. At the end of this time each was isolated in a vial which thereafter was examined daily for eggs. At the end of a two-day period the insects were again placed together for two days in order to increase the possibility of fertilization. They were then reisolated for two days. This procedure was repeated until eggs were laid or until death resulted. Of these one hundred individuals only seventy laid eggs. The remaining ones were counted as males, although some may have been sterile females.

The egg laying process was peculiar. The female would stretch her body backwards as far as possible without losing her foothold. Soon afterwards, exuding from the genital aperture, the egg appeared as a shapeless mass which assumed a spherical form and hardened in a few seconds. She lowered her abdomen until the sticky egg adhered to a particle of soil and then she pulled away from it. She turned, stroked the egg once or twice with her antennae, nibbled at it and then left it without further care. This process corresponds to that of another springtail, *Sminthurus viridis* (L.) as described by Davies (1928). Females usually laid an average of eleven eggs a day and then there ordinarily followed a period during which no eggs were laid. The average total number of eggs laid by one female was forty-five.

HATCHING

The eggs remained in contact with the soil until hatching. As development took place, the average diameter of the eggs increased from 0.16 mm. to 0.19 mm. (Plate I, 9-10).

The hatching insects split the chorion by extending and flexing the body. As soon as the legs were freed they were used for jerking the rest of the body out of the shell. In dry conditions it was not unusual to see an emerging springtail with empty shell dragging behind, attached to the furcula. The newly emerged insects averaged 0.56 mm. in length (Plate I, 11, 1). Other springtails would nibble at the exposed parts of the body when it was still moist. No setae disappeared and the young one did not seem to be harmed while this "caressing" was taking place.

ECDYSIS

Before ecdysis, or molting, took place, the body turned an opaque white color. The insect usually attached its feet to the soil and moved the thorax up and down until the old skin began to split along the mid-dorsal region of the thorax. This continued until the thorax, head, antennae and

first two pairs of legs were freed, which required about ten to twenty minutes. After resting, without moving, for about five minutes, the animal attempted to free the rest of the body. Others nibbled at the new skin as they did during the hatching process. In fact, some of the springtails were observed to peel back some of the old skin to expose a fresh area. This seemed to hasten the shedding. The springtails would sometimes free themselves of the exuviae by crawling through the soil.

The whole moulting process normally would be completed within an hour (Plate I, fig. 8).

RESULTS OF EXPERIMENTS

THE LENGTH OF THE EGG STAGE IN LOW HUMIDITY

Experiments were undertaken to determine whether a low or high humidity was more conducive to the hatching of the eggs. The temperature used throughout these tests was 30° C.

In the first tests, eggs were left in 100 per cent relative humidity. The average length of time required for hatching was six days. Ninety-four per cent hatched.

Other eggs were placed in 90 per cent relative humidity. Sixteen days were required and only 50 per cent hatched. Of this number very few of the nymphs were able to extricate their entire bodies and the ones which did so died shortly afterwards. No eggs hatched when left in 80 per cent relative humidity.

THE LENGTH OF EGG STAGE AFTER REMOVAL FROM LOW HUMIDITIES

Eggs were placed in low humidities for five days and then removed to 100 per cent relative humidity at 30° C. Figure 2 shows graphically the results of these experiments. The graph indicates that as relative humidity decreased the length of the egg stage increased, while the percentage of hatching decreased. Below 50 per cent relative humidity the eggs ceased to be viable.

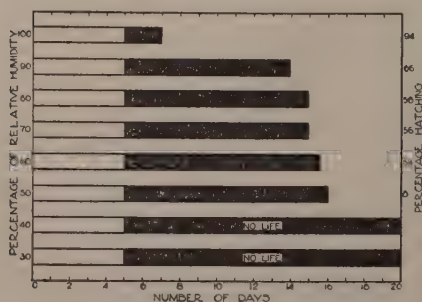


Fig. 2. An analysis of the effect of drying on the length of time required for development in the egg stage.

In figure 2 the unshaded areas represent the length of time the eggs were subjected to the various relative humidities. The solid black areas indicate the length of time required for hatching.

THE LENGTH OF EGG STAGE AT VARIOUS TEMPERATURES

Ten vials, each containing ten eggs, were placed in constant temperature boxes and subjected to temperatures of 25° C., 30° C., 35° C., and 36.7° C., each group being kept in 100 per cent relative humidity. The results are tabulated in

figure 3. One can see that of the temperatures used, 30° C. approaches nearest the optimum for development. At this point the percentage hatching was higher and the length of time required for hatching was shorter than at any other. Lower and higher temperatures lengthened the period of development.

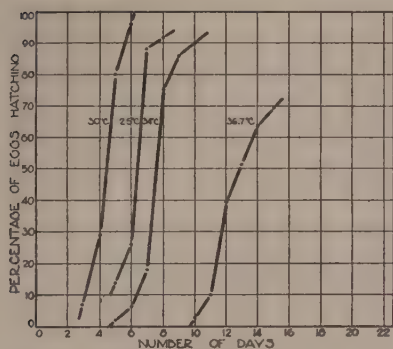


Fig. 3. Hatching curves at various temperatures.

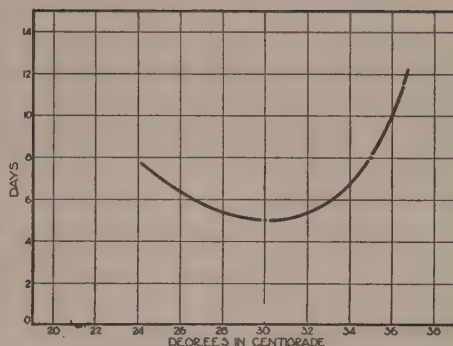


Fig. 4. Time-temperature curve for development of eggs of *P. violenta*. (Each point represents the average of 100 eggs.)

Figure 4 pictures in a more condensed form the averages of the results shown in figure 3. In this graph one can see that 30° C. approaches nearest the optimum for development of the eggs of the temperatures used.

THE LENGTH OF TIME REQUIRED FOR NYMPHAL DEVELOPMENT AT DIFFERENT TEMPERATURES

In this experiment four hundred newly hatched nymphs were used. Ten of these were allotted to each vial, and ten vials, or one hundred nymphs, formed one group. These four groups were subjected to temperatures of 25° C., 30° C., 34° C., and 36.7° C., respectively. Since it is difficult to determine accurately when a springtail is mature, the period required for maturity was measured from the time of hatching until eggs appeared in the vials. The results are shown in figure 5.

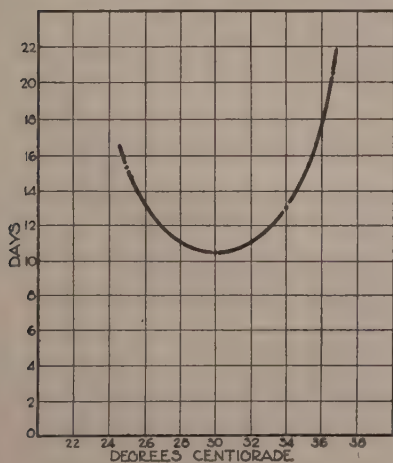


Fig. 5. Time-temperature curve for the development of nymphs of *P. violenta* from hatching until maturity. (Each point represents the average of 100 individuals.)

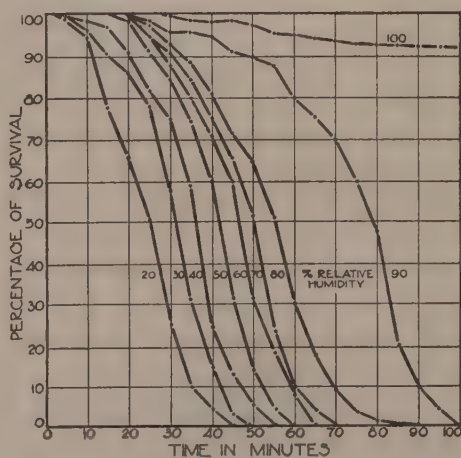


Fig. 6. Percentage of survival in various humidities.

LENGTH OF INSTARS

An attempt was made to measure the living insects at daily intervals, but they were so very active that it was impossible to keep them in the focus of the microscope long enough to make the required measurements. Just enough anaesthetic was given to make them sluggish, but this had a harmful effect on them. They were not as active afterwards and they lived but a few days. These two methods were therefore discarded, and the following method was used.

A large number of eggs was placed in a vial at 30° C. and, as they hatched, the nymphs were changed to other vials. Complete data were kept on the ages of these insects. Twenty-five of them were taken out every day as a sample, and the widths of their heads were measured after the insects had been killed in alcohol. In table 1 the results are shown tabulated to Dyar's law (1890).

Maclagan (1932) found that the lucerne flea, *Smynthurus viridis* (L.), has seven nymphal instars and an imago. The writers found that *Pseudosinella violenta* (Fols.) has only six instars and an imago (Plate I, 1-7). The width of the head was used for the measurements and a calibrated oculater micrometer scale was used in a compound microscope for measuring.

TABLE 1. *Measurements of instars*

| Observed width in mm. | Ratio of Increase | Theoretical width in mm. (calculated from Dyar's factor, 1890) | Instar |
|---------------------------------|-------------------|--|--------|
| .12 | | | 1st |
| .14 | 1.166 | .12 × 1.175 equals .141 | 2nd |
| .18 | 1.289 | .141 × 1.175 equals .165 | 3rd |
| .21 | 1.111 | .165 × 1.175 equals .194 | 4th |
| .25 | 1.190 | .194 × 1.175 equals .228 | 5th |
| .28 | 1.120 | .228 × 1.175 equals .268 | 6th |
| .33 | 1.178 | .268 × 1.175 equals .315 | Imago |
| 1.175 average ratio of increase | | | |

The observed widths were reasonably close to the calculated widths. The greatest difference was 0.3 mm.

The average length of time spent in each stadium is shown in the following table.

TABLE 2. *Duration of stadia (30° C., 100 per cent relative humidity)*

| Instar | I | II | III | IV | V | VI |
|----------------|---|----|-----|----|---|----|
| Time (in days) | 1 | 1 | 1 | 2 | 2 | 3 |

More time was spent in the sixth stadium, probably due to the sexual development at this period.

THE LENGTH OF LIFE OF ADULTS IN DIFFERENT HUMIDITIES

The insects were subjected to percentages of relative humidity from 100 down to 20 per cent. Units of one hundred individuals were used for

each humidity. These one hundred were divided into groups of ten in a vial without soil for convenience in counting (figure 6).

Observations were made at five minute intervals and a careful record was kept of the number of deaths in each container. It is to be observed that there is a distinct difference between the number surviving in 100 per cent and in all relative humidities below 100 per cent.

SUMMARY

A special technique is described for handling the small soft bodied insects. A modified "aspirator" was used.

Observations show that positive thigmotropism is stronger than negative phototropism.

The sex ratio of one hundred individuals taken at random in the Insectary greenhouse was 70:30, females and males, respectively. One hundred offspring of five females were examined and thirty-five were males.

Copulation was not observed at any time.

Other individuals were seen to "graze" on the moisture on the surface of hatching nymphs and on moulting springtails.

There were six nymphal instars.

Experiments showed that:

1. One hundred per cent relative humidity is the most favorable for hatching and development.
2. Thirty degrees Centigrade was the optimum temperature of those used in the experiments.
3. At 30° C. and 100 per cent relative humidity the length of the egg stage was five days. The nymphal stages lasted ten and one-half days in the same humidity and temperature.

When eggs were placed in low relative humidities for five days and then removed to optimum conditions, the length of the egg stage was increased and the viability was decreased.

The adults required a one hundred per cent relative humidity or had to go to a saturated soil frequently in order to thrive.

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PLATE I

EXPLANATION OF PLATE

1. First instar nymph.
2. Second instar nymph.
3. Third instar nymph.
4. Fourth instar nymph.
5. Fifth instar nymph.
6. Sixth instar nymph.
7. Imago.
8. Third instar nymph undergoing ecdysis (molting).
9. Newly laid eggs.
10. Eggs just before hatching, seen from various angles.
11. Two hatching nymphs. The upper figure shows a nymph emerging, posterior end first, and the lower figure shows a nymph emerging, anterior end first.

PLATE I



MITOSIS OF CIRCULATING CELLS IN THE HEMOLYMPH OF THE ROACH, *BLATTA ORIENTALIS*

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Most researchers who have made more than cursory examinations of insect body fluids record observations of indirect division among the circulating amebocytes found in the hemolymph. Among these workers are Cuénot (2), Metalnikov (6), Poyarkoff (11), Barrott and Arnold (1), Hollande (4), Paillot (10), Müller (8), Muttkowski (9), Iwasaki (5), Miall and Denny (7), Yeager and Tauber (17), Tauber and Yeager (12), Yeager, Shull, and Farrar (15), and others listed by Tauber (13) in a survey of the more important publications dealing with this subject. Taylor (14) has also noted the occurrence of karyokinesis in cultures of hemolymph cells taken from another roach, *Periplaneta americana*. Haber (3), alone, reports negatively on *Blatella germanica*, the croton cockroach: "During my investigations I have not seen mitosis although I have rather closely examined several hundred smears."

Few reports on the quantitative values for the number of dividing cells in insect hemolymph have been made. Paillot (10) found that the normal mitotically dividing cell occurrence was from three to four per thousand cells in immature *Euproctis chrysorrhoea*. Iwasaki (5) counted one to two mitotically dividing cells per thousand in the hemolymph of normal *Galleria mellonella* larvae. Yeager and Tauber (17) state that the average mitotically dividing cell (M. D. C.) count was found to be 0.51 per cent, or approximately five karyokinetic cells per thousand cells counted, in the hemolymph from *Periplaneta fuliginosa*, a semi-tropical roach. This paper reports the values of M. D. C. counts in hemolymph samples taken at various intervals from isolated specimens of *Blatta orientalis*.

MATERIALS

Specimens of the roach, *Blatta orientalis*, were collected either in Ames, Iowa, or were shipped in from collectors in Kansas or Mississippi. No attempt was made to keep the different groups separate; all insects captured or sent to Ames were placed together in large stock cages. Fresh water and food, in the form of whole-wheat bread and well-ripened banana, were supplied regularly. Before being used for counts, all specimens were allowed to remain in the stock cages for several weeks of acclimatization to laboratory conditions. Insects intended for counts were selected randomly from the stock colony, placed in a glass jar, and kept under close observation for an additional ten to fourteen days while given access to the usual stock diet. All sluggish or injured individuals were removed. A second period of observation followed when individual specimens were isolated in 125 cc. Erlenmeyer flasks and observed for another week before actual determinations began. Small amounts of the customary food were supplied each animal in its individual container.

Since no separate source of water was available, the pieces of bread were well moistened before being placed into the flasks. The mouth of each container was covered with cheese-cloth held in place by a rubber band. Each insect was transferred daily to a clean flask and a new supply of food. To prevent too rapid drying of the food, the small flasks were kept in a large, loosely covered glass aquarium.

To dilute the hemolymph and to stain the cells in the temporary mounts prepared for the mitotic counts, the following stock solution was made: to one liter of distilled water were added 4.7 grams sodium chloride, 0.11 gram potassium chloride, 0.11 gram calcium chloride, and 0.005 gram gentian violet. This was heated gently until the dye completely dissolved. When prepared for use, 0.25 cc. of 10 per cent acetic acid was added to 20 cc. of the stock solution. The acid prevents cell coagulation and fixes the cell protoplasm so that structures in the stained amebocytes are more easily visible.

To allow use of an oil immersion lens and to prevent drying of the diluted hemolymph preparation, the clear mineral oil, known commercially as "Nujol," was employed.

TECHNIQUE

To make the hemolymph cell counts the following technique, modified from Yeager and Tauber (17), was used: Upon a clean glass slide was placed a large drop of mineral oil. Into this drop was placed a small drop of the diluting-staining solution described above. This droplet was made to settle to the top of the slide. A tiny sample of antennal hemolymph (obtained by clipping with scissors) from the insect was quickly placed into the colored solution and immediately stirred with a dissecting needle to distribute the cells throughout the droplet. No coverslip was used, but to prevent evaporation, the diluted, stained hemolymph was covered with a film of the surrounding mineral oil, which also thus served as immersion oil for the microscope lens. The preparation was not disturbed for several minutes to allow the cells to settle to the slide. Cells were then counted randomly, using oil immersion of approximately 1,000 diameters magnification. As the count progressed, the number of cells in prophase, metaphase, anaphase, and telophase was noted. Experience and tests showed that a count of two thousand cells was sufficient to obtain an accurate figure for the percentage of dividing cells. Determinations made on counts of less than two thousand are likely to be erroneous. A count of 2,500 or more produces results of no significantly greater accuracy than those from two thousand cells.

RESULTS

Two groups of animals were used during the course of these researches. Group I of thirty specimens was made of individuals kept different lengths of time, varying from ten days to 132 days. M. D. C. counts were made at various intervals—sometimes daily, sometimes every 48 hours, sometimes every 72 hours, and so on, but rarely was a period as long as five days allowed to elapse between counts. Data from this group are contained in table 1. Group II was made of 125 animals kept for five days only. Three counts were made on each roach: on the first, third, and fifth days. Summaries of pertinent data from these counts are given in table 2.

TABLE 1. *Summary of mitotically dividing cell (M.D.C.) percentages from thirty animals in Group I*

| Animal number | Description | Counts made | Days observed | Cells counted ($\times 10^3$) | M.D.C. range (%) | M.D.C. average (%) |
|------------------------------------|-------------------------|-------------|---------------|---------------------------------|------------------|--------------------|
| 621 | ♂ Nymph | 25 | 48 | 50 | 0.00 — 0.40 | 0.166 |
| 623 | " | 29 | 71 | 58 | 0.00 — 0.45 | 0.162 |
| 727 | " | 25 | 29 | 50 | 0.15 — 0.35 | 0.250 |
| 728 | " | 17 | 22 | 34 | 0.05 — 0.45 | 0.229 |
| 621,623,727,728 | " | 96 | 170 | 192 | 0.00 — 0.45 | 0.203 |
| 614 | ♂ Adult | 19 | 38 | 38 | 0.10 — 0.30 | 0.205 |
| 625 | " | 28 | 72 | 56 | 0.05 — 0.40 | 0.194 |
| 725 | " | 20 | 33 | 40 | 0.05 — 0.30 | 0.160 |
| 614,625,725 | " | 67 | 143 | 134 | 0.05 — 0.40 | 0.186 |
| 619 | ♀ Nymph | 73 | 132 | 146 | 0.00 — 0.50 | 0.202 |
| 620 | " | 31 | 63 | 62 | 0.05 — 0.45 | 0.225 |
| 624 | " | 17 | 42 | 34 | 0.05 — 0.40 | 0.211 |
| 723 | " | 24 | 26 | 48 | 0.05 — 0.35 | 0.137 |
| 619,620,624,723 | " | 145 | 262 | 290 | 0.00 — 0.50 | 0.194 |
| 622 | ♀ Adult | 26 | 48 | 52 | 0.05 — 0.40 | 0.238 |
| 721 | " | 17 | 26 | 34 | 0.05 — 0.35 | 0.188 |
| 722 | " | 25 | 32 | 50 | 0.05 — 0.40 | 0.222 |
| 622,721,722 | " | 68 | 106 | 136 | 0.05 — 0.40 | 0.216 |
| 751 | Sex and age not checked | 10 | 11 | 20 | 0.05 — 0.30 | 0.140 |
| 750 | " | 19 | 23 | 38 | 0.00 — 0.30 | 0.176 |
| 747 | " | 11 | 13 | 22 | 0.10 — 0.35 | 0.200 |
| 745 | " | 13 | 16 | 26 | 0.00 — 0.40 | 0.238 |
| 744 | " | 14 | 20 | 28 | 0.05 — 0.40 | 0.203 |
| 742 | " | 16 | 19 | 32 | 0.05 — 0.40 | 0.193 |
| 741 | " | 15 | 19 | 30 | 0.00 — 0.40 | 0.216 |
| 737 | " | 9 | 11 | 18 | 0.05 — 0.30 | 0.155 |
| 733 | " | 17 | 27 | 34 | 0.05 — 0.35 | 0.200 |
| 731 | " | 12 | 13 | 24 | 0.05 — 0.25 | 0.133 |
| 730 | " | 14 | 19 | 28 | 0.05 — 0.35 | 0.153 |
| 729 | " | 10 | 10 | 20 | 0.00 — 0.30 | 0.155 |
| 726 | " | 11 | 12 | 22 | 0.05 — 0.20 | 0.127 |
| 724 | " | 9 | 14 | 18 | 0.05 — 0.20 | 0.116 |
| 675 | " | 9 | 12 | 18 | 0.00 — 0.30 | 0.133 |
| 613 | " | 3 | 12 | 6 | 0.10 — 0.20 | 0.150 |
| All roaches of unknown sex and age | " | 192 | 251 | 384 | 0.00 — 0.40 | 0.172 |
| All roaches in Group I | — | 568 | 932 | 1136 | 0.00 — 0.50 | 0.186 |

Certain facts in table 1 are particularly important and, for convenience and clarity, need emphasizing. Altogether 568 counts of 2,000 cells each were made on the thirty roaches. The range of individual counts extends from 0.0 per cent to 0.50 per cent; the average M.D.C. count is 0.186 per cent with a range from 0.116 per cent (animal No. 724) to 0.250 per cent (animal No. 727). Averages for male nymphs, male adults, female nymphs, and female adults are, respectively, 0.203 per cent, 0.186 per

cent, 0.194 per cent, and 0.216 per cent. The average for all nymphs is 0.198 per cent; for all adults, 0.201 per cent; for all males, 0.195 per cent; for all females, 0.203 per cent.

Table 2 shows that the range of individual counts from the second group of 125 animals is also from 0.0 per cent to 0.50 per cent. The average from all the initial counts is 0.196 per cent; the average from all counts within the period of five days is 0.181 per cent.

TABLE 2. *Summary of data from 125 animals in Group II*

| Number of Animals | Description of count | Counts made | Days observed | Cells Counted ($\times 10^3$) | M.D.C. range (%) | M.D.C. average (%) |
|-------------------|---------------------------------|-------------|------------------------|---------------------------------|------------------|--------------------|
| 125 | Initial count | 125 | 0 | 250 | 0.00 — 0.45 | 0.196 |
| 125 | Counts on 1st, 3rd and 5th days | 375 | Each animal for 5 days | 750 | 0.00 — 0.50 | 0.181 |

Figure 1 consists of drawings of dividing cells in various stages. It will be noted that cells of different sizes were found to be engaged in the karyokinetic process. No effort was made to differentiate the mitotic cells so far as cell types or classifications were concerned.

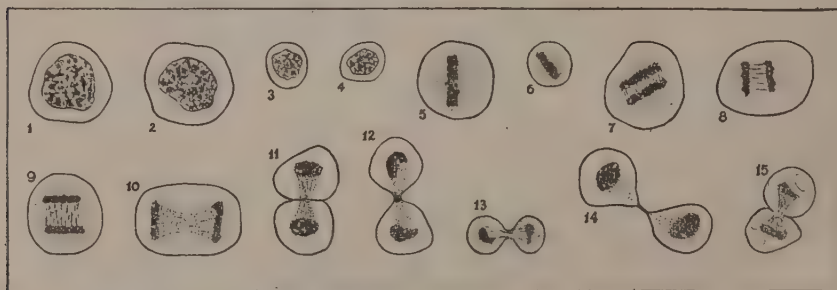


Fig. 1. Sketches of dividing cells found in roach hemolymph. 1, 2, 3, 4: early pro-phases of large and small cells; 5, 6: metaphases; 7, 8, 9: early anaphases; 10: late anaphase; 11, 12, 13: telophases; 14: two daughter cells about to separate; 15: an abnormally vacuolated cell in early telophase. (1,000 x)

Figure 2 graphically shows the course of the percentage of M.D.C. counts for animal No. 619, which was kept under observation the longest length of time, namely, 132 days. The range of percentage is from 0.0 per cent (that is, less than one dividing cell per two thousand cells counted) to 0.50 per cent. This range happens to be the widest encountered in any of the roaches in the first group. The average M.D.C. count from 75 samples is 0.202 per cent.

The course of counts from three animals, No. 723, No. 727, and No. 750, was selected for figure 3 to check the possibility that changes in laboratory temperature might be a variable affecting the normal mitotic rate. Since counts were made any time between 7:30 a. m. and 10:00 p. m., and since the temperature was noted at each count, a fairly good check on

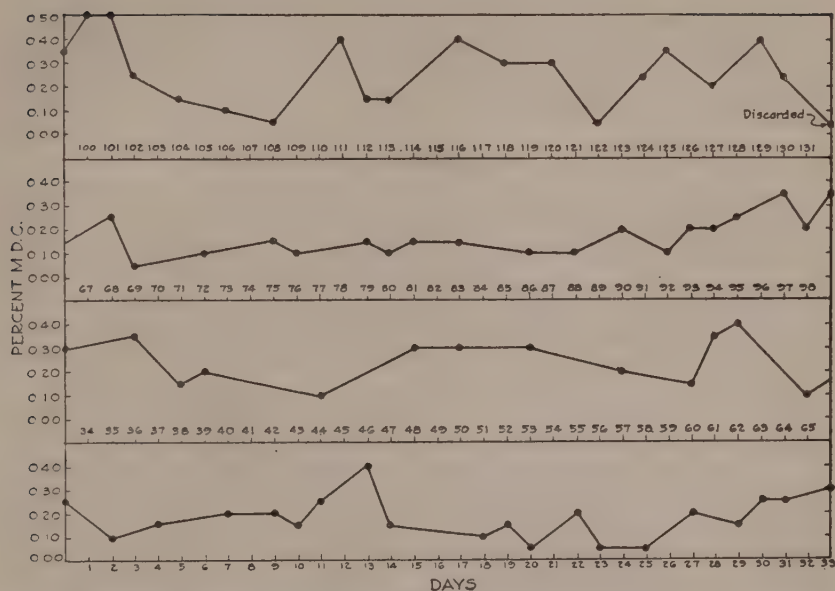


Fig. 2. Mitotically dividing cell (M.D.C.) percentages from animal No. 619 kept under observation for 132 days.

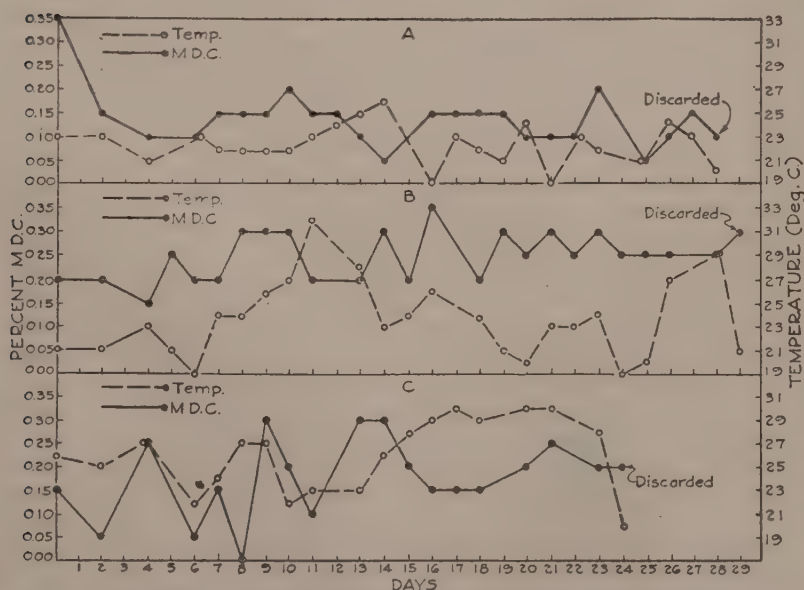


Fig. 3. Comparison of mitotically dividing cell (M.D.C.) percentage curves from animals No. 723 (A), No. 727 (B), and No. 750 (C) with laboratory temperature curves for the duration of observation.

room temperature fluctuations was obtained. The observations were made during a period of more than a year, consequently the range of temperatures is rather wide, namely, 17° to 32° C. No correlation between variations in the percentages of M.D.C. counts and fluctuations in room temperature is found.

DISCUSSION

Considering the large amount of data collected from 155 animals by means of 943 samples of hemolymph, it seems quite evident that the normal specimen of *Blatta orientalis*, be it male or female, large nymph or adult, is quite likely to have a mitotically dividing hemolymph cell count somewhere within the limits of 0.0 per cent to 0.50 per cent, when based on a count of 2,000 cells. Counts of 0.0 per cent or 0.50 per cent are rare; the former occurring 20 times, the latter only three times in the 943 determinations.

The extent of the range of average counts, from 0.116 per cent to 0.250 per cent, might lead one to suspect some factor or factors of influencing individual insects. However, high and low averages were found, indiscriminately, among males, females, adults, and large nymphs, with no connection to the sex or age factors mentioned. Fluctuations in room temperature were also without apparent effect. Thus it seems that a high or low count is a specimen characteristic allied to individual variation, or is a response to controlling factors which have thus far not been isolated.

A significant feature to notice in tables 1 and 2 is connected with these figures: the average M.D.C. value of 0.186 per cent from the first group of thirty animals; the average of 0.196 per cent from the initial counts of the second group of 125 animals; and the average of 0.181 per cent from the 375 counts made on the same group of 125 roaches. The close agreement of these data provides a three-way check on the average M.D.C. count value, which can be said to lie, approximately, within the limits of 0.180 per cent to 0.200 per cent; the mean is about 0.190 per cent.

As yet no explanation can be given for the changes in the M.D.C. percentages from day to day. One male nymph was kept for 71 days; another, 48 days. A male adult was studied for 72 days. Two female nymphs were kept for 132 and 63 days, respectively; a female adult for 48 days. In only a few cases were the same M.D.C. percentages repeated from one count to the next. These specimens were in good condition as long as the counts were continued. Evidently the methods of handling, feeding, caging, and counting were satisfactory. Moreover, their continued good condition and their M.D.C. counts which remained within the limits of 0.0 per cent to 0.50 per cent, are indications that the slight necessary hemorrhages concomitant with taking of hemolymph samples, in themselves, did not produce any superficially evident damage to the insect, nor any harm or effect which might be reflected in the hemolymph picture. In a few cases among the 155 roaches studied the observations were terminated by death from undetermined causes. Usually, the taking of "normal" M.D.C. counts was stopped by certain physiological conditions such as ecdysis or ovipositions, whose effects on the hemolymph picture will be reported in a later paper.

So far as indicated by these studies, the M.D.C. values seem unrelated to age if restricted to large nymphs and active adults. No investigation was made of the counts at different instars, but in view of the changes that occur at molting, when the animal undergoes a period of rapid growth, it may be that younger roaches may have average M.D.C. percentages differing from those reported here.

In classifying insect hemolymph cells some workers [See Tauber (13) for a review of this literature] have placed emphasis on the occurrence or non-occurrence of mitosis as a characteristic for separating cell types. Observations made in connection with this paper gave no results which might indicate that indirect division was confined to any particular class of amebocytes. All types which normally are present in the hemolymph stream seemed to divide karyokinetically. To classify cells from the hemolymph of *Blatta orientalis* other distinguishing differences will need to be utilized.

The presence or absence in insects of definite, fixed hemolymph cell producing organs or tissues has also been a matter of dispute among certain of the leading workers already referred to. Since no histological proof has yet been presented to show the presence of a real hematopoietic organ, supporters of the belief that there is such an organ somewhere in the insect body point to the low percentage of mitotic cells, which can be found in the circulating hemolymph, as an indication that those divisions would be too few to replace cells lost through age, disease, cytolysis, or other means. However, if the following assumptions are made, an interesting argument can be presented against that view:

1. That the hemolymph volume of a large nymph or an adult *Blatta orientalis* is about 30 cubic millimeters. [Yeager and Tauber (16) found that the total hemolymph volume of *Periplaneta fuliginosa*, a slightly larger roach, was approximately 35 cubic millimeters.]

2. That the hemolymph cell population in *B. orientalis* is about 32,500 per cubic millimeter. [Tauber and Yeager (12) found that the average hemolymph cell count from 125 specimens of this species was 32,858 cells per cubic millimeter.]

3. That about 0.2 per cent of the hemolymph cells are dividing. (Approximately the average figure reported in this paper.)

4. That a free, ameboid cell, such as a hemolymph cell in an insect, when ready to undergo mitosis, can complete the division within three hours. (Most cytology texts state that a division of a cell of this type is completed in about one hour.)

Then, by simple calculation, it can be determined that within a three-hour period at least 2,000 cells might be produced in the normal roach for the purpose of maintaining hemolymph cell population. Under certain physiological and pathological conditions this rate of replacement might be increased. Certainly it cannot be denied that the cells resulting from mitotic division in the hemolymph stream contribute considerably to formation of new cells in the body fluid, even if there is present, in addition, an hematopoietic organ of some sort.

CONCLUSIONS

1. Based on a total of 943 hemolymph samples of 2,000 cells each from 155 normal, large nymphs or active adult specimens of the roach,

Blatta orientalis, this insect has a normal mitotically dividing hemolymph cell count which ranges from 0.0 per cent to 0.50 per cent for individual sample determinations and from 0.116 per cent to 0.250 per cent for individual animal averages. Individual sample counts of 0.0 per cent or 0.50 per cent are rare. The general average is approximately 0.19 per cent.

2. Although the count varies from day to day because of unknown reasons, the determined percentages are stable enough to fall within the normal limits and seemingly are not affected by age, sex, or fluctuations in laboratory temperature.

3. With methods described, specimens can be maintained for over 130 days in good condition. The slight hemorrhages necessary to obtain hemolymph samples are not reflected in any changes in the hemolymph picture, so far as the karyokinetic cell population is concerned.

4. Mitosis does not seem to be confined to any one or several types of cells. So far as could be determined in this work all kinds normally present undergo indirect division.

5. Cells resulting from karyokinetic division in the circulating medium of the roach contribute (perhaps without assistance from any fixed hematopoietic organ) to the maintenance of a total hemolymph cell population by the replacement of cells lost through age, disease, cytolysis, or other means.

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NATIVITY OF CUCURBITA MAXIMA¹

A. T. ERWIN

From the Vegetable Crops Subsection of the Iowa Agricultural Experiment Station

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In a recent bulletin by Erwin and Haber (4), the conclusion was expressed that two of the economic species of pumpkins—*Cucurbita pepo* and *C. moschata*—are native to North America, but the evidence as to the nativity of the third species, *C. maxima*, “remains an open question” and requires further consideration. In this paper consideration is given to a review of the evidence to date of *C. maxima* being indigenous to North America.

Gilmore² (6) stated that *C. maxima* “is found in tropical and sub-tropical North America. . . . To the Southwest, whence came the crop plants of aboriginal culture in Nebraska, the remains in ruins sometimes reveal the identity of plants of ancient culture there. The occurrence of squash seeds in some of the mortuary bowls is important, indicating the ancient use of this vegetable for food.” He also directs attention to the fact that the term squash is a familiar one among the Indians. Gilmore (7) reports also *C. maxima* as one of the species identified by him in the vegetal remains of the Bluff-Dwellers culture in the Ozarks.

Densmore (3) in a “List of Plants Used as Food by the Chippewas” includes *Cucurbita maxima*, but he submits neither evidence nor authority for the statement.

Gilmore’s conclusions found apparent support from two sources. The term squash is of Indian origin and finds expression in their ancient religious rites; moreover, the name squash or Patun is a familiar family name among the Hopi. Historically the term squash as applied to plants by the Indians is generic rather than specific in its application, and as noted by Bailey (1), “The term squash does not have a botanical implication, but historically it was probably associated mostly with forms of *C. pepo*.” Secondly, Gilmore (6) reported the identification of *C. maxima* in vegetal remains recovered in archeological surveys made in the Southwest. The allusion in this case, though not stated, is apparently to the findings of Waugh (8). The report by Waugh (8) of the determination of *C. maxima* from southwestern United States is the only record found by the writer.

Through the courtesy of Curator S. J. Guernsey the author secured for examination the Waugh specimens bearing the Peabody Museum label No. A1252. These seeds, Mr. Guernsey advises us, were found in a jar in a cliff-house cave in Sayodneechee Canyon in northeastern Arizona. The seeds embrace two types. The majority of the specimens are *C. moschata*. The fimbriated margin of a darker color than the body clearly identifies them as this species. The second type is not typical *C. moschata*. The mar-

¹ Journal Paper No. J351 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 298.

² Plate 28 of this paper shows pictures of two varieties of *C. maxima*, with the subtitle “Varieties of squashes and pumpkins cultivated by tribes of Indians of Nebraska from immemorial time.”

gin is not definitely fimbriated but has the appearance of a thread-like mass being matted down and both the body and the border are of the same shade of light brown color. The opinion of the writer is that the character of the margin precludes the possibility of this type being *C. maxima*, and the truncate instead of oblique seed scar lends support to this point of view³. The lack of a more clearly defined margin may be attributed to the manner of gathering the seeds while immature. Numerous other specimens of peduncles, rinds and seeds have been recovered in this general region, but no evidence of *C. maxima* has appeared.

Under the leadership of Mr. Mark Harrington of the Museum of the American Indian, the remains of a pre-Columbian culture have been discovered in the Ozarks of southwest Missouri. Below these remains he found a stratum of still older level, designated by him as the Bluff-Dweller culture. Similar and even more extensive explorations have been made covering the same geological formation in northwestern Arkansas under the direction of Prof. S. C. Dellinger of the University of Arkansas. These explorations have brought to light a considerable quantity of well-preserved vegetal remains. Gilmore (7), who has studied widely in the field of ethnobotany, in commenting on the Bluff-Dweller material, states "that even though the rock shelters are situated in a region of fairly humid climate, their physical structure is such that the exteriors of most of them are extremely dry . . . so that the organic remains have been perfectly preserved." In these collections are found numerous specimens of cucurbits, and in the list of plants determined by Gilmore, *C. maxima* is included. Through the courtesy of Dr. Dellinger the writer was afforded opportunity to study the Arkansas Bluff-Dweller material at the University of Arkansas, where it is now stored. Well-preserved specimens of seed, rind and pedicels of cucurbits from numerous localities are found in this collection, all of which so far observed by the author are *C. pepo*; however, it was disappointing that no specimens of *C. maxima* were found in the Ozark Bluffs material⁴.

In southern Mexico, cucurbits abound. In the primitive regions the blossoms, rind and seed are a staple article of food and are used in innumerable ways strange to us. This region is regarded by many students as the probable home of several species of cucurbits. In a visit to this territory during the winters of 1934 and 1936, the writer was impressed by the fact that in the more remote sections all of the varieties observed belonged to either *C. pepo*, *C. moschata* or *C. ficifolia*. Next in importance to maize as a food, cucurbits of some variety were found to be used everywhere. In numerous village markets were observed specimens of cucurbits, none of which were *C. maxima*. Likewise, cucurbit seed offered for sale in these markets were examined with similar results.

Roasted pumpkin seeds are eaten in many parts of Mexico much as peanuts are in this country. Some varieties of *C. maxima*, such as King of Mammoth, produce seed fully one-half larger than in other species of

³ Since this paper was written, specimens were submitted to L. H. Bailey, who identified them as *C. moschata*. Letter to author under date of Dec. 12, 1935.

⁴ Since the above article was written, the author is in receipt of a letter under date of Nov. 21, 1935, from Dr. Gilmore, in which he advises that, having made a more extended study of this material, . . . "*C. maxima*, I do not find in the Ozark Bluff-Dweller remains."

pumpkins and hence are particularly adapted for roasting; but the writer was unable to locate any of this species so used.

This survey was made in a trip by auto, covering 1,000 miles, and many interior villages were visited, including the extensive public market at Oaxaca. Mexico is a country of numerous valleys and such a wide range of climate that any generalizations as to crops needs to be made with extreme caution. However, all efforts to locate *C. maxima* in the primitive regions of Mexico were wholly negative. This observation as regards Mexico coincides with the statement of Zhiteneva (9), who, in discussing the nativity of this species, says, "*C. maxima* occupies South America, Peru and Bolivia, not transgressing to the North."

An examination by the writer, of plant material recovered from mortuary bowls from the archeological explorations at Metla and Monte Alban under the direction of Dr. Alfonse Caso, failed to reveal *C. maxima*, though the preservation of this material has been affected by the presence of moisture. In the National Archeological Museum of Mexico are found clay models of pumpkins (natural size), classed as belonging to the ancient civilization of the Tarrascans, and also incense burners ornamented with pumpkin flowers, which specimens are identified by Dr. Guillermo Gandara (5) as *C. pepo* and *C. moschata*. The significant fact should be noted that *C. maxima* is not represented. However, Dr. Gandara regards the pipian pumpkin, a pre-Cortesian food plant of Mexico, as a cross between *C. maxima* and *C. moschata* and therefore deduced that if pipian existed, *C. maxima*, one of its progenitors, must have also existed; a hypothesis in which the writer does not concur, because the studies (4) made of species hybrids of cucurbits show clearly that such crosses evidence pronounced manifestations of impotence.

Wittmac regards *C. maxima* as of South American origin and reported to De Candolle (2) that seeds recovered from the tomb of Ancon, near Lima, Peru, had been "certified by M. Daudin as belonging to this species," and Zhiteneva (9) apparently shares this point of view.

The writer is in receipt of historic cucurbit seeds from Professor Juilio C. Tello⁵, bearing the label *C. maxima*. These specimens are highly suggestive of *C. maxima*, but because of their age major portions of the testa had crumbled away, making exact determination difficult. Further archeological explorations, particularly in Peru, may bring to light additional cucurbit material, including pedicels, which would assist materially in determining the nativity of this species. So far as North America is concerned, the evidence to date is, this writer thinks, wholly negative in character and points to the conclusion that *C. maxima* is not indigenous to this country.

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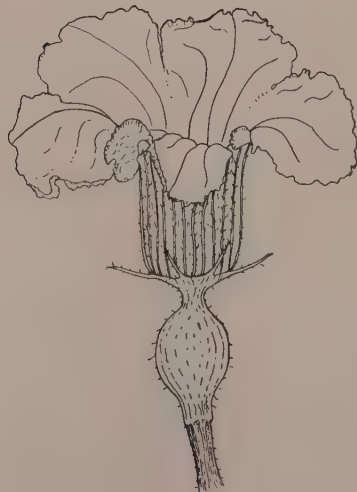
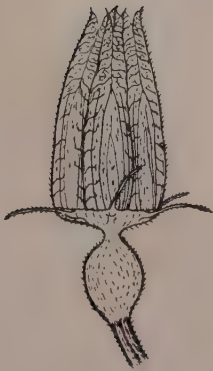
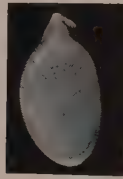
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PLATE I

Typical leaf, pedicle, seed, bud, and pistillate flower of *Cucurbita maxima*.

PLATE I



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